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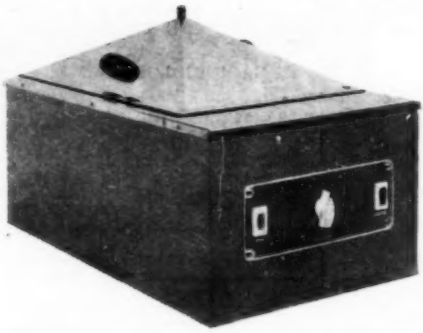

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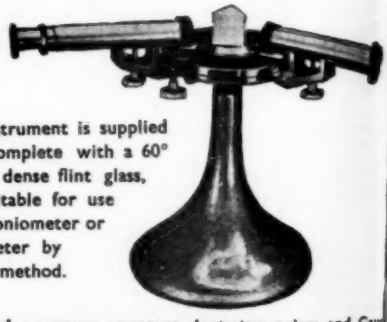

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EXCELLENCE AND LEADERSHIP IN A DEMOCRACY

THE need to foster outstanding qualities among democratic peoples has formed the theme of a series of articles in *Daedalus*. A number of draft papers were prepared and considered at a closed conference held at Middletown, Connecticut, during April 28–30. The final papers have now been brought together and published in the *Proceedings of the American Academy of Arts and Science* (**90**, No. 4; Autumn 1961). Among the papers is one by Robert S. Morrison, director of medical and natural sciences at the Rockefeller Foundation, who discusses the need for new types of excellence.

Morrison began his inquiry by means of an informal survey among a representative group of friends. Almost all found time to formulate thoughtful analyses of future needs. This fact serves to document an awareness among intellectual leaders for something different and a good deal better than now exists. All seemed well satisfied with the progress being made in expanding knowledge of the physical world and in applying that knowledge to the reduction of poverty and suffering. Most of present pre-occupation is with organization, interdisciplinary co-operation, decision making, and value problems. Science is increasingly confronted with large problems the parts of which are so interdependent that they cannot be dissected without spoiling them for future study. A contribution by Warren Weaver shows how the scientific problems of the future merge without perceptible transition into the classical problems of the humane arts.

"I have a very strong conviction", he wrote, "that the 'new forms of excellence' required by science over the next half-century will in considerable part consist of techniques for dealing with broader problems, larger problems, problems which arise when a moderate number of small problems are organically interrelated."

One can start with the very obvious and oversimplified examples. "The day is past", Dr. Weaver claims, "when science can properly treat food supply as an isolated problem, birth-rates as another, public health as another. The whole body of servomechanism and feedback theory; many of the techniques of operations research; the emerging knowledge concerning information and communication theory—these are all groping toward the capacity to deal with problems of organized complexity. . . .

"We have been good at producing bits of information, fragments of knowledge. We now need to devote more attention to the fitting together of these bits, to broaden interrelations, and hence to broader utilization. We have to humanize science, and give more general meaning and structure—and even value—to it."

Most of the other correspondents to Dr. Morrison's inquiry came directly to the need for synthesizing, interdisciplinary research, better organization, and better decision-making, without bothering to analyse how. All recognize that it is the extraordinary dimensions of this pile which shape the demand for new forms of excellence.

It is science which has: (1) given so much information that no man can master more than a small portion of it; (2) given reality to the concept of one world; (3) given the populations of some parts of that world so many material possessions and so much power over their own destinies that for the first time the paramount question is not: "How do I get enough to stay alive?" but "What do we do with a life that is more or less guaranteed?"

Each gift calls for its own sort of expertize, and, taken together, requires a form of human activity so new that there is no word to describe it. The rapid accumulation of raw information and digested knowledge places unprecedented burdens on the minds and characters of those who follow the learned professions. It is not cheering to know that this year's class of new medical students are confronted with four times as much to learn as their teachers encountered a short twenty years ago. By the time these young men finish their courses they should return immediately to the basic sciences they studied as freshmen. There will be as much that is new to them as there was when they studied the basic sciences the first time.

It is not only the possession of specialized bits of knowledge that sets a specialist off from his fellows but also his different way of looking at things, and this may erect even more serious barriers. Whole schemes of thought and discourse have developed to deal appropriately with the findings provided by separate skills, until ultimately the specialities are divided from one another not only by the possession of special information and manipulative skills but also by wholly different ways of thinking and talking. Nowhere is this more clear than in the field of psychology. Here there are psychoanalysts, learned theorists, conditioned-reflex addicts, physiological psychologists and ethologists, all looking at the same set of phenomena in such wholly different ways that they can scarcely hold sensible conversations with one another.

The unsatisfactory nature of this state of affairs is widely recognized and lies behind the persistent cries for more 'interdisciplinary' research and better 'generalists'. How does one set about becoming excellent in a general way? If there is substance to the view that specialization arises from the invention of special methods of observation and is perpetuated

by human incapacity to store and recall more than a small fraction of our exponentially increasing pile of data, it seems reasonable to give primary attention to simplifying these functions or transferring them to other hands. Our best minds might then become freer to deal with conceptual and communication problems which worry us so much.

There seems reason to hope that many of the basic scientific specialities are less separated by technique than they used to be. The electron microscope was invented by physicists; but methods for its use have been worked out by chemists, cytologists, histologists, microbiologists, and an occasional physiologist. It is beginning to give a view of biological work which is clearly more unified than anything before. Now that instruments exist for analysing events at what is essentially molecular level, the attention of all specialities is beginning to focus at the same point. As a result, the histologists, biochemists and physiologists are now holding livelier conversations with one another than at any time. It is increasingly difficult to differentiate the basic science departments on the basis of the instrumentation used within them. Basic biochemical apparatus, electron and light microscopes, cathode-ray oscillographs, and radiation counters may well be found in any of them. Part of the increasing homogeneity is due to the recognition that all biological events can be traced to biochemical ones. This means, in turn, that all biologists must learn to speak the *lingua franca* of biochemistry—and ultimately of atomic physicists, at least in so far as the nature of the chemical bond between atoms is concerned.

The splitting of our scientific culture into language blocks does not doom man to the splendid isolation of the 'Tower of Babel'. The separate ways, if pursued long enough, can lead to an even more splendid and imperial synthesis of crystal clarity. It is possible for a single individual to select and give a meaningful synthesis to the most significant facts and concepts of economics, sociology, history, business management, and political science—*vide* W. W. Rostow, *The United States in the World Arena*. As knowledge expands beyond a certain point, the burden on the individual can become less. For it is much easier to grasp and to remember interrelated schemes in which a set of observations developed in one language can be interpreted in terms of another than it is to hold on to a mass of unrelated items.

It also seems probable that the pursuit of excellence in the technology of gathering and processing data will help to free mankind from the burdens of specialization. In the early stages of any natural science, the great advances come from devising new ways of observing. Recent technical advances have reduced many of the essential manipulative skills to semi-automatic mechanical processes. There is now, for example, a machine which will automatically analyse a complex protein for all its constituent amino-acids in a matter of hours. Only a few years ago whole teams of skilled chemists were trying to find out how the job could be done at all.

The second problem—how to handle the data after they are accumulated—is more difficult. It remains for the future to devise an excellence which will make the most of the speed and accuracy with which mechanical computers handle large volumes of data, while at the same time maximizing the associative capacities and instinct for relevance contained within our own brains. Enough progress has been made to suggest that computer techniques will be useful not only in storing and recalling existing information, that is, in improving the library function. There are great possibilities for the development of machines which will analyse and correlate data as they occur. For example, neurophysiologists and psychologists are now much interested in the possibility of correlating electrical events in the nervous system with functions such as perception, learning and memory.

New forms of excellence are required for developing these new technologies for the accumulation and analysis of data. More interesting, perhaps, is the challenge the new techniques offer for the development of even newer excellences. It will not be easy to relinquish to the machine the arts and techniques which have taken decades to master, and to concentrate on the even more demanding tasks of generalization, synthesis and judgment which only men can carry out. Perhaps the greatest demand for ingenuity will fall on the field of education, once it is decided to hand over to other means the mere memorization of information, the computing of sums, and such other routine intellectual tasks as may be found appropriate. It will be an excellent teacher who shows how to programme school and college experience so as to maximize the capacity to grasp general principles, detect relevance where none was seen before, and create new conceptual schemes. When that day comes, all education will be general education since the technical problems which now divide the specialities will in large part have been handed over to the computers, and "we will have taught ourselves a language we can all use in common".

Dr. Morrison also examines the set of problems arising from the emergence of "one world". When radiation fall-out affects both the just and unjust of all peoples in the world, it is time to invoke Waddington's concept of 'stochastic' or 'statistical morality' which calls for a new kind of excellence that must be invented to deal with this new state of affairs. The whole future of the race is at stake in such matters as the uncontrolled expansion of its numbers in the 'population explosion', or the decline in its quality brought about by the genetic effects of advances in modern medicine in combination with our present-day casual breeding patterns.

There is a school of thought, particularly well exemplified by the tobacco industry, which attempts to shrug off the evidence for all such dangers as 'only statistical'. At present the evidence available suggests that men who smoke a package or more of cigarettes a day are likely to die earlier than men who do not smoke at all. Yet smoking habits have little changed. The explanation seems to lie in the

fact that men still find it very difficult to pay serious attention to contingencies that lie more than a few years in the future and are coupled to present events with a probability of less than one.

After examining the need for improving standards of government, Dr. Morrison shows how compulsive are the mass uses of indoctrination techniques. There is great need for responsible people to be constantly aware of the dangers inherent in specialities and in propaganda machinery. There is need for a combination of the highest sort of intellectual and moral attainments. In the first place, it should be unnecessary in a democracy to point out that a speaker has no business to try to make the final decision for his hearers. This would be, in fact, the simple way out and is common procedure in dictatorships, theocracies, and other controlled societies. Democracy rests on the assumption that people in general will reach the right decision if they are given the truth. But the truth is impossible to define in abstract terms, and even in concrete matters like the effect of radiation on genetic anomalies, is not much more than a fluctuating area of agreement.

The telling of this kind of truth cannot be guaranteed by constitutional articles or perjury laws. As more and more features of our life fall under the control of those who wield a specialized body of knowledge, control measures must be sought. These can be found only in the individual moral and intellectual excellence of those who develop, hold, and disseminate knowledge.

THE HISTORY OF SCIENCE

The Search for Order

The Development of the Major Ideas in the Physical Sciences from the Earliest Times to the Present. By Cecil J. Schneer. Pp. xvii + 398. (London: English University Press, Ltd., 1960.) 21*s*. net.

THIS book belongs to the class, of which there have been several examples of late, of bird's-eye views of the history of physical science from early times to the present. The chief defect of this type of work is that it inevitably omits everything but the direct ancestry of the science of our time, and makes what is in fact an almost indescribably devious course, constantly doubling back on itself, appear like a straight line. Of its kind, however, it is among the best. The story is told in a clear, straightforward and interesting manner, and, apart from an excessive number of small inaccuracies that could well have been avoided, the subjects dealt with are on the whole presented faithfully. Those subjects include, mainly, physics, with some chemistry and a brief glance at geology and evolution. Despite a picture of a spiral nebula on the dust cover, there is no cosmology and next to no astronomy.

There are a few surprising passages. For example, the conflict between physicists and geologists over the age of Earth is presented as a conflict between "historical knowledge" and ordinary physical knowledge, as though these were different in kind, instead of as an instance of the different conclusions that can be drawn from two incomplete sets of data. On a smaller scale, while it is certainly true that J. J. Thomson "lived on into the electronic era", the essence of the fact scarcely appears in that expression of it; and there are numerous quotations of which the authorship is either not given or indicated ambiguously.

But the chief criticism to which the book is open concerns its underlying attitude, which, though it appears overtly only here and there, can be felt throughout—the attitude, namely, that identifies present knowledge, so far as it goes, with truth. Whatever is is right, and ever will be, and whatever else was is wrong, and will never be again. We are told, for example, that "the [relativity] theory must for ever remain . . . ", and that "the assertion of the unity of all living things through the theory of evolution, is contradicted only by the ignorant or the intellectually vain". What one should learn from the history of science is that the fact that one is living now is a mere accident, and that the science of our time will appear to a historian of the future as crude as that of the seventeenth century appears to us, while some of the latter crudities (the transmutability of elements has already done so) may then have returned to displace our present superstitions. The present time is an utterly undistinguished point in an indefinitely long sequence; it is not the culminating moment "for which the floating forceless ages waited". Unfortunately, few historians of science make this their central theme.

HERBERT DINGLE

PSYCHICAL RESEARCH

William James on Psychical Research

Compiled and edited by Gardner Murphy and Robert O. Ballou. Pp. viii + 339. (London: Chatto and Windus, Ltd., 1961.) 30*s*. net.

CONDITIONS in New England around the mid-nineteenth century were such as to throw up a number of people of exceptional talents. Among them were the brothers James—William, investigator of the paranormal, and Henry, the novelist. We are concerned here with the former, the elder of the two by one year. They had much in common, especially a marked streak of pragmatism. Its manifestations were different, practical and empirical for William, introspective and self-observational for Henry. So we find William taking Medical School at Harvard, and receiving an invitation to teach physiology to students, which soon led, as one would expect, to the borders of psychology and the associated problems of the mind.

William James on Psychical Research deals with the last forty years (1870–1910) of William's life by presenting his papers, combined with an illuminating introduction and retrospect. The collection is valuable, more perhaps on account of the personalities involved than for the content of the contributions themselves. As a commentary on the state of psychical research, and indeed of much advanced philosophical thought of the period, it is admirable. But if the reader is to enjoy these pages as he should—and as they deserve to be enjoyed—he might try the experiment of overlooking a good deal of the descriptions of séances, media, occult happenings and so on—which naturally aroused a fair amount of suspicion and controversy—and concentrate on the courage and sincerity of the people in question. We need pass no verdict on what they did, or what they thought it all meant: what stands out is a monument of intellectual valour.

XUM

James was always asking "Is it really so?". Herein lay his strength, devoted as he was to his friends and their common purpose. Not always could he go the whole way with them.

Seen against the background of the *fin de siècle*, one is struck by the power for good of Anglo-American co-operation: the foundation in 1882 of the Society for Psychical Research in England with a 'cell'—later a full society—in the United States.

In Great Britain the movement got away to a flying start with the active support of such figures as Henry Sidgwick, Edmund Gurney and Frederic Myers, not to mention Lord Rayleigh. Sidgwick's critical powers and Gurney's passion for hard work were essential, but the genius of the group was undoubtedly Myers, his life (largely) a tragedy and his death heroic. To his contemporaries he was a literary and historical scholar of exquisite sensibility, a Fellow of Trinity College, Cambridge, who, having no love for teaching, became an inspector of education. But as some will recollect his name as the author of the great poem *St. Paul*, the question can scarcely be avoided: What is the relation between these verses and his intense search for the nature of the survival of personality after death? We may never know, except to be humbled by the magnitude of the endeavour, for he was not one to count the cost or heed the wounds. Indeed, as Myers himself wrote: "Desperate patience of a brave desire."

An event of consequence, not mentioned by the compilers, was the short but brilliant life of the Metaphysical Society (1869–80). Among its members were Shadworth Hodgson, who founded the Aristotelian Society, and G. C. Robertson, who initiated *Mind*. Both those were closely connected with the Society for Psychical Research, having no doubt sharpened their wits and deepened their perception in discussions aforetime with such giants as Cardinal Manning, Father Dalgairns and Dean Stanley.

Meanwhile, James continued to press for experimental evidence. We gain little information as to how this was answered. There was much checking of mediumistic phenomena, trances and so on, but surely something more directly physical could have been attempted, even if the results were negative. It is worth remembering that the Einthoven string galvanometer appeared in 1903, and made possible an enormous increase in sensitivity for the measurement of minute electrical effects. Seemingly, comparatively little was done by direct instrumental means, or deliberate laboratory controls.

Prof. Gardner Murphy in the retrospect already mentioned, and writing about 1958, refers to four accomplishments of William James which make him a leading character in the history of psychical research. These are: (i) he gave the subject the necessary prestige, (ii) he set things well and truly on their feet in the United States, (iii) he kept facts well to the fore, and (iv) he discovered the famous Mrs Piper as a wholly exceptional medium. Now, long after the event, these do not appear to be very striking achievements, but in the intellectual climate of the time they counted for much.

But they were symptomatic of a fundamental quality. Prof. R. B. Perry wrote that James always "Knew there was more", and that is the outlook of the born explorer. Somebody once said to James, "the great field for new discoveries is always the unclassified residuum". (How forcibly indeed this was illustrated, for example, in the discovery of argon by Rayleigh and Ramsay.) Evidently it must be a basic tenet for students of the paranormal, from whatever direction they may approach it.

Nevertheless, it was left to Myers to pose the elemental question: "Is the Universe friendly?". We cannot tell. What matters is that this timely volume shows William James and his circle following the light as they saw it, and leaving behind them a cherished example of dedicated research.

F. I. G. Rawlins

CHEMISTRY IN INDUSTRY

Ammonia

Manufacture and Uses. By Dr. A. J. Harding. Pp. xiii + 41 + 7 plates. (London: Oxford University Press, 1959. Published under the auspices of Imperial Chemical Industries, Ltd.) 6*s*. 6*d*. net.

Electrolytic Manufacture of Chemicals from Salt

By Dr. D. W. F. Hardie. Pp. xii + 74 + 7 plates. (London: Oxford University Press, 1959. Published under the auspices of Imperial Chemical Industries, Ltd.) 7*s*. 6*d*. net.

THE books under review are the second and third in a series which is being published by Imperial Chemical Industries, Ltd., with the assistance of the Science Masters' Association and the Association of Women Science Teachers. The aim of the series is to help correlate academic chemistry as taught in schools, colleges and universities with chemistry as it is applied industrially.

Dr. Harding's book on ammonia has chapters on its manufacture, its properties and technical data, and its uses. There is also a bibliography and a short appendix on the application of physical chemical principles. The first chapter is by far the longest: it is concerned with the sources of the raw materials, the purification of hydrogen and nitrogen, and the actual synthesis process itself. Apart from the underlying chemistry, the mechanics and economics of the processes are briefly described.

Dr. Hardie, in his discussion of the salt industry, has chapters on the fundamentals of salt electrolysis, raw materials and energy, the manufacturing processes, packing, storage and transport of the products, production statistics and trends, physical and chemical properties of the products, principal uses of the products, and an outline of historical development. In addition, there are a bibliography, a brief list of important dates in the development of the industry, and a glossary.

Both volumes deal with their respective subjects clearly and in a way that should appeal to sixth-form student, university undergraduate and graduate alike. The authors bring home to the non-industrial chemist the interplay of chemistry, engineering and economics which is such an important part of industrial chemistry. Perhaps the most valuable service to the academic chemist is the way in which the authors show how the production of one particular chemical is co-ordinated with that of so many others in modern chemical industry. An example of this, quoted from Dr. Hardie's book, is that of chlorine production. Electrolytic chlorine production must go hand in hand with caustic soda production (sodium production is of relatively minor importance). However, recently the demand for chlorine for the production of chlorinated organic compounds has outstripped the demand for caustic soda. Hence investigations have been

made into the aerial oxidation of the hydrochloric acid which is produced as a by-product of organic chlorination processes. The academic chemist finds to his surprise that the obsolete Deacon and Weldon processes are likely to be resuscitated on economic grounds.

These two books are well illustrated with line diagrams and plates. The authors and publishers are to be congratulated on these books which keep up the high standard set by the first volume in this series: *Sulphuric Acid: Manufacture and Uses*, published by the Kynoch Press for Imperial Chemical Industries, Ltd. in 1955. M. J. NEWLANDS

ISOTOPES IN HÆMATOLOGY

The Use of Isotopes in Hæmatology

By L. G. Lajtha. Pp. x+83. (Oxford: Blackwell Scientific Publications; Springfield, Ill.: Charles C. Thomas, 1961.) 21*s*. net.

THE application of isotopic techniques to hæmatological problems is standard procedure in modern medicine. All too often their use has assumed the significance of a status symbol. Estimation of blood and plasma volume, the mean life-span of the erythrocyte and the absorption of radioactive cobalamin are procedures of established value. There is therefore a place for an authoritative account covering these and related topics.

The first two chapters of Dr. Lajtha's book describe the estimation of plasma and red cell mass and the estimation of the mean cell life of the erythrocyte. The next two are concerned with radioactive vitamin B_{12} and iron, and there are two short chapters on white cell and platelet tagging and autoradiography.

The section on autoradiography is undoubtedly the best and reflects the author's vast experience in this field. The technique of preparing autoradiographs is set out in precise detail and the description is warmly recommended to anyone proposing to work in this field. The other sections are less happy. The description and comment may well meet the need of the general hæmatologist who looks for guidance in setting up and interpreting tests involving the use of isotopes, but is unlikely to please the expert in the various fields.

Surface counting techniques are mentioned in somewhat general terms, but a detailed account of how one should set about doing them and how to interpret the results is absent. The estimation of mean cell life using chromium-51 is always of interest in a hæmolytic anæmia, but occasionally information about the site of destruction of the red cells may provide decisive information as to whether splenectomy is indicated or not. Simultaneous iron-59 and chromium-51 studies, the former providing the additional information about the site of red cell formation, are often performed together and perhaps should have found a place in this book.

Equally important is the emphasis the author places (or fails to place) on various procedures. Both an *in vitro* and *in vivo* method for labelling red cells with chromium are described and little guidance is given as to which is the method of choice.

Similarly, four methods of estimating absorption of vitamin B_{12} labelled with cobalt-58 are listed and discussed, namely: the fæcal excretion test, the urinary excretion test, the hepatic uptake test and the plasma test. The change in plasma radioactivity after an oral dose of vitamin B_{12} does not merit consideration as a routine test of absorption of vitamin B_{12} even if material of sufficiently high specific activity were available. It certainly has no place as a screening procedure. On the other hand, many would regard the hepatic uptake of vitamin B_{12} labelled with cobalt-58 after an oral dose in a far more favourable light than does Dr. Lajtha. It is independent of the ability and willingness of the patient to collect his fæces (? for 15 days) or urine.

Finally, a section devoted to the behaviour of the limited range of isotopes of use in the hæmatological laboratory and to the equipment that the hæmatologist could most profitably invest in in order to count these isotopes would have greatly enhanced the value of the book. I. CHANARIN

ZOOLOGICAL LITERATURE FOR 1958

The Zoological Record

Vol. 95 : Records of Zoological Literature relating chiefly to the year 1958. Edited by G. Burder Stratton, assisted by Marcia A. Edwards. Pp. v+2234. (London: Zoological Society of London, 1961.) 160*s*.

THE present volume of *The Zoological Record*, relating chiefly to the literature of 1958, is bigger than ever, and provides good evidence, if this were necessary, of the great expansion of the subject in recent years. The *Record*, apart from being a necessity to anyone engaged in taxonomic studies, also furnishes a good indication of the amount of work being carried out in the different groups of the animal kingdom. In addition, the valuable system of classifying publications according to subject enables one to see the relative attention being devoted to the various aspects in each of the eighteen groups of animals listed. A study of the Insecta, by far the largest section, comprising 660 pages, shows an increasing number of publications dealing with physiology and ecology, and in the subject index the number of pages under each heading is as follows: insect morphology 9, development 12, physiology 21, bionomics 10, ecology 19, evolution 8, geographical and faunistic 27. The main divisions show little difference from those in recent years: Mollusca, with 187 pages, is followed by Protozoa 147, Pisces 141, Vermes 131, Mammalia 125, Arachnida 123, Aves 112, Crustacea 110, Amphibia 100, Reptilia 93, Trilobita 86 and in gradually diminishing size to Porifera 7. The number of new genera and subgenera is nil in Protochordata, one in Amphibia, and reaches a maximum of 949 in the Insecta. It is noteworthy that the total number of new genera and subgenera has shown no diminution in recent years and as evidence of the amount of taxonomic work in progress does not support the pessimistic views of certain authorities concerning the dearth of taxonomists.

This is the last volume to be issued in bound form. Starting with Volume 96 (literature for 1959) the separate sections will be sent to subscribers as soon as they are published; a title-page and binding case will be forwarded when the volume is complete. This will bring information of current research to users of the *Record* more quickly. In addition, the format, beginning with Volume 96, will be changed to crown octavo, to give the larger type area necessary for recording the expanding volume of literature each year. EDWARD HINDLE

XUM

Elsevier's Dictionary of Automation, Computers, Control and Measuring in Six Languages

English/American, French, Spanish, Italian, Dutch and German. Compiled and arranged on an English Alphabetical base by W. E. Clason. Pp. vi+848. (Amsterdam : Elsevier Publishing Company ; London : D. Van Nostrand Company, Ltd., 1961.) 140*s*.

THE increase in technical and scientific knowledge has occasioned a crying need for multilingual technical dictionaries. Bilingual technical dictionaries (many of which are of indifferent standard) are common enough; multilingual dictionaries are less common. The present *Dictionary* is one more attempt to make good the deficit.

There are 3,390 entries with an excellent index in each of the five non-English languages giving quick access to any of the entries in any of the languages used. Clearly, because of the extent of the scientific field covered, there must be a priority among the entries selected since it is not be to expected that 3,390 entries could cover such a number of highly specialized fields. As with all choices, the present one cannot be expected to meet with wholesale approval or to be everyone's taste and must inevitably be a function of the compiler's bias. Nevertheless I question why, for example, there are no entries for 'electronic counter', 'core', 'obey', whereas there are entries for "Westphal balance" and "mobilometer". On the other hand, it is only fair to point out that one welcomes the (unexpected) entries "tape travel" and "pigeon hole pocket". One might comment at random in this way but it does not detract from the fact that this appears to be an excellent addition to the long line of Elsevier multilingual dictionaries covering other scientific fields. Its price may place it outside the average scientific pocket, but it will certainly be of help in the libraries of scientific and industrial research laboratories.

The printing, binding and lay-out of the work are of the high standard and quality one has long come to expect from Elsevier. S. WHELAN

Practical Microscopy

By Dr. C. L. Duddington. Pp. ix+237. (London: Sir Isaac Pitman and Sons, Ltd., 1960.) 30*s*. net.

Histological and Histochemical Technics

By Prof. Harold A. Davenport. Pp. xxii+401. (Philadelphia and London: W. B. Saunders Company, 1960.) 49*s*.

Section Cutting in Microscopy

By Dr. H. F. Steedman. Pp. vi+172. (Oxford: Blackwell Scientific Publications; Springfield, Ill.: Charles C. Thomas, 1960.) 21*s*. net.

SO many books on microscopy and microscopical technique have appeared in the past few years that new publications on these subjects may seem unwarranted. All three books reviewed here, however, seem to have much to justify their publication. Dr. Duddington has addressed his work to the serious amateur, and to the elementary student of biology. He assumes no previous scientific knowledge, and introduces the reader in turn to the component parts of the microscope and to some of the basic techniques of microscope manipulation. The second part of the book consists of an introduction to the methods of preparing botanical, zoological and bacteriological specimens for investigation. There is a short bibliography, listing most of the standard reference works on the subject, some of which may, however, prove too technical for those who have only just taken up this type of study. Perhaps a future edition might grade the references and indicate those likely to prove most useful for elementary students. The book is well produced and clearly illustrated.

Histological and Histochemical Technics, by Prof. Davenport, is designed for the advanced worker, and is concerned entirely with methods of preparing specimens. It provides a survey in some detail of the processes of fixation, embedding and staining, including an extensive discussion of their more theoretical aspects as well as the actual formulæ. One useful chapter describes techniques of staining in the block, a method of preparation which seems to attract comparatively little interest at the present. The final chapters are devoted to a brief survey of histochemical methods, and would serve as a good introduction and source-book for those who use such methods only occasionally.

Section Cutting in Microscopy, by H. F. Steedman, is a book largely for the expert. It presents details of all the usual methods of microtomy, in addition to describing several new techniques developed by the author. The approach is logical throughout and the author has made a most commendable attempt to rationalize some of the more common microscopical techniques. It is pleasing to see the inclusion of a section on possible faults which may present themselves during the cutting of paraffin sections. Other chapters deal very fully with the use of ester waxes, the water-soluble waxes and with the use of urea formaldehyde resin. There is also a chapter on the preparation of ultra-thin sections for electron microscopy. This book is a welcome addition to the reference works which one expects to find in every histological laboratory. S. BRADBURY

The Theory of Crystal Structure Analysis

By A. I. Kitaigorodskii. Translated from the Russian by David and Katherine Harker. Pp. xi+275. (New York : Consultants Bureau, Inc., 1961.) 12.50 dollars.

IN the preface of this book, which appeared in the original Russian version in 1957, the author makes it clear that he is describing his subject-matter up to the year 1955. It is perhaps a tribute to the rate of development in this field that many of the author's analyses and opinions seem either outmoded or bizarre at the present time. In particular, the analyses concerned with distribution functions have been repeated more formally and elegantly in the past few years ; the author also dismisses anomalous dispersion as of 'no importance' and the difference synthesis as 'devoid of interest' (this even drew a translators' comment).

However, much of the book is devoted to the basic theories of the Patterson method, intensity statistics and direct methods and, for these, lack of topicality is of less consequence. The treatment of these topics is such as will commend itself either to the crystallographer of mathematical inclinations or to the mathematician with some knowledge of crystal structure analysis. The section dealing with inequality relationships is of particular interest, being concerned, to a large extent, with the author's own excellent work. The book reads well, which is symptomatic of the quality of the translation.

To summarize: a work of considerable interest to those able to appreciate it and one the main faults of which are due to the lapse of time since it first appeared. M. M. WOOLFSON

THE ROYAL SOCIETY

ANNIVERSARY ADDRESS*

By Sir HOWARD FLOREY, F.R.S.

AWARD OF MEDALS, 1961

Copley Medal: Sir Hans Krebs, F.R.S.

SIR HANS KREBS has made outstanding contributions to knowledge of the chemical pathways of metabolism. In 1932, with Henseleit, he used tissue slices to demonstrate the synthesis of urea and found that additions of ornithine stimulated the process. Ornithine was regarded as uniting with carbon dioxide and ammonia to give arginine, with citrulline as an intermediate. The enzyme arginase liberated urea and a molecule of ornithine, so that the process could begin again. Subsequent research has shown that the postulated cycle is essentially valid, although more detail has been introduced; carbamyl phosphate, argininosuccinate and adenosine triphosphate have been brought in and aspartic acid has replaced ammonia in the reaction with citrulline. The more complicated version owes much to the fundamental work initiated by Krebs in 1937 on the tricarboxylic acid cycle. This process is now seen as the main route for oxidizing the two-carbon fragment produced in the biological degradation of carbohydrates, fatty acids and amino-acids.

The initial acceptor of acetyl coenzyme *A* is oxalacetate and the completion of the cycle is marked by regeneration of a molecule of oxalacetate, and by the oxidation of one C_2 fragment to carbon dioxide and water. The product of the interaction of oxalacetate and acetyl coenzyme *A* is citric acid, and the later steps in the cycle result in the liberation of carbon dioxide and pairs of hydrogen atoms. These latter finally unite with oxygen to form water but the intermediate stages are coupled with the formation of adenosine triphosphate from adenosine diphosphate. The Krebs cycle does not allow for the net synthesis of the intermediates, which are themselves important in providing the carbon framework for many biosyntheses. The main cycle therefore needed to be reinforced by postulating ancillary mechanisms leading to the synthesis of available four-carbon intermediates. Krebs's work on the glyoxylate cycle showed that iso-citrate, by yielding succinate and glyoxylate, permitted the formation of malate by the interaction of acetyl coenzyme *A* and glyoxylate. The malate led directly to the oxalacetate needed by the main cycle, and the succinate could be used as a precursor or could permit the replacement of intermediates drawn out of the cycle by synthesis of cell substances.

The very complex overall picture is both useful and elegant. Nobody deserves more credit for this than Krebs.

A Royal Medal: Prof. C. F. Powell, F.R.S.

Prof. Powell is distinguished by his outstanding contributions to our knowledge of cosmic rays and of the fundamental particles.

He started research under Prof. C. T. R. Wilson and made important contributions to the problem of condensation of water vapour to form drops at elevated temperatures. He then worked with Prof. A. M. Tyndall at Bristol on the mobility of positive ions in gases. This was a difficult subject, bedevilled by the effects of impurities, and Tyndall and Powell raised it to new levels of definiteness and reliability.

About twenty years ago Powell turned his attention to the development of the use of photographic techniques for studying the interactions and other properties of fast particles. Up to that time such techniques were rarely used but, through collaboration with those concerned with the production of photographic emulsion, Powell established a new and powerful technique which ever since has played a most important part in the study of energetic particles.

The first applications were to the study of nuclear interactions, but quite soon the technique was applied to investigate cosmic radiation. This soon resulted in the discovery with Occhialini of the π-meson by analysis of the tracks of cosmic ray particles in emulsions exposed at high altitude in the Bolivian Andes. Further major discoveries followed from the work of the laboratory at Bristol which was so enthusiastically led by Powell. These included the τ-meson and evidence for the decay of neutral mesons into γ-radiation. Improvements in emulsion technique to increase the rate of detection of fast electrons resulted in the recent observation of multiple meson production in nuclear encounters.

Apart from these outstanding individual discoveries, Powell and his collaborators have made a great number of significant contributions to many aspects of cosmic ray and fundamental particle studies. They have introduced new and improved techniques whenever the scope of existing techniques has become inadequate. Thus they developed the use of balloons to carry emulsions to high altitudes for cosmic ray studies and they introduced the emulsion stack which made it possible to follow the tracks of very fast particles, right through to their end-points.

With the introduction of artificial accelerators capable of providing beams of particles of energy comparable with the main bulk of cosmic ray particles, Powell and his group have turned part of their attention to fruitful use of emulsion stacks to study, in more detail than hitherto, the properties of mesons and hyperons. They have, however, taken up as well the difficult problem of studying the ultra-high-energy cosmic radiation and already have obtained information of interest.

Powell has not only made many outstanding personal contributions to this work but has been, through his enthusiasm and organizing ability (which has involved large-scale international co-operation), a continual source of inspiration to everyone concerned. The pioneering work at Bristol has been, and will continue to be, of the greatest importance in

* Delivered at the Royal Society on November 30.

the physics of high-energy phenomena and of the fundamental particles.

A Royal Medal: Sir Wilfrid Le Gros Clark, F.R.S.

Sir Wilfrid Le Gros Clark is internationally known for his work in two apparently unrelated fields, namely, neuro-anatomy and primate evolution. However, an important feature of his neurological work has been the way in which he has used the evidence of comparative anatomy to shed light on the evolution of the human brain. His work on the connexions of the thalamic nuclei with the cortex and the hypothalamus is especially noteworthy in this respect. He and his collaborators have studied the interrelationships of various parts of the brain using in particular methods depending on retrograde and transneuronal degeneration. He was able to show that following a small retinal lesion, localized patches of atrophy appeared in the lateral geniculate body and by these means he mapped the projection of the retina on the lateral geniculate body and on the cortex. Other investigations of this type have added considerably to our knowledge of the olfactory system and have removed misconceptions concerning the relationship between the hippocampus and the rhinencephalon.

Le Gros Clark is generally recognized as a leading authority on primate evolution. His early work on the anatomy and behaviour of *Tarsius*, based on painstaking personal observations in Borneo, is a model of its kind. During the past fifteen years he has played a leading part in the examination of many important fossil discoveries, especially in Africa. His critical assessment of the Australopithecinae and the tentative conclusion that they may be closely related to the ancestral stock from which the Hominidae are derived, is one of the landmarks in this branch of anthropology. He was also closely associated with the unmasking of the Piltdown forgery, thus helping to remove a puzzling discrepancy from discussions on human evolution.

Apart from these major fields of work Le Gros Clark has contributed much to general anatomical and biological problems. His work on muscle regeneration, for example, showed elegance and clarity, and as honorary director of the Medical Research Council Ergonomics Research Unit he has supervised research in a new and expanding field and has given a new impetus to applied anatomy.

Davy Medal: Prof. D. H. R. Barton, F.R.S.

Prof. Barton's researches have for the most part been concerned with the chemistry of natural products, a field which has provided him with numerous opportunities to demonstrate his brilliance in the elucidation of complex structures, the unravelling of stereochemical complications, and the elaboration of syntheses. He has always taken every opportunity to make good use of physical methods in solving problems of organic chemistry, and much of his earlier work on the applications of differences of molecular rotation, particularly in the terpene and steroid fields, has had far-reaching effects on our knowledge of stereochemical relationships. His pioneering work on conformational analysis in alicyclic systems has revolutionized concepts of the relationship between stereochemistry and reactivity. More recently he has been studying long-range effects, by which, for example, a remote ethylenic linkage can exercise a marked influence on the condensation reactions of a carbonyl group in a triterpene system.

His notable contributions to the establishment of the structure and intricate chemistry of caryophyllene have been followed by fruitful investigations on many other sesquiterpenes, such as tenulin, pyrethrosin, lactucin, and geigerin, while in the di- and tri-terpenoid series partial or complete structures have been established for a substantial number of natural products; these include the citrus bitter principle, limonin, the structure of which had defied elucidation for a considerable time, and clerodin, also a bitter principle. His investigations on steroids and their derivatives have been prolific, and include some particularly elegant work on cevine and related steroid alkaloids. The biogenetic mechanisms by which natural products are built up from simple precursors in Nature have interested him for some time, and studies on these processes led to a simple two-stage synthesis of usnic acid from methylphloracetophenone.

The most remarkable results of his researches have probably been those arising from his investigations on the photochemical transformations which sometimes occur when compounds are irradiated with ultra-violet light. The classical work carried out more than sixty years ago on the irradiation of santonin gave some indication of the problems involved in studying such reactions, but Barton has succeeded in establishing the structures of the three products, isophotosantonic acid lactone, photosantonic acid, and lumisantonin, and has explained mechanistically the remarkable ways in which these unusual compounds are derived; he has shown, furthermore, that other *cyclo*hexadienones, such as prednisone, behave similarly. Quite recently he has used photochemical methods to bring about an attack, by a group in a favourable stereochemical position, on a non-activated carbon atom, and by most brilliant applications of this method he has achieved spectacular syntheses of aldosterone acetate (in three stages from corticosterone acetate) and of 19-noraldosterone acetate. Perhaps even more remarkable is his beautiful synthesis of conessine, in which the heterocyclic ring involving C_{18} is formed by photolysis of a steroidal C_{20} azide.

The brilliance and originality of Prof. Barton's work has gained him an international reputation and his laboratories at the Imperial College of Science and Technology have become a rendezvous for research workers from many countries.

Sylvester Medal: Prof. P. Hall, F.R.S.

In his first two papers on finite group theory, Prof. Hall broke new ground in the fields of soluble groups and groups of prime-power order. In 1928 he established the validity of soluble groups of some striking extensions of the classical theorems of Sylow, thereby opening a new epoch in the study of finite soluble groups. Five years later he laid the foundation for all subsequent work on groups of prime-power order with a long and systematic paper, the many original and fruitful ideas of which included that of the regular p-group. In the years between the appearance of these landmarks in the history of group-theory and the War he published many other papers on groups of finite order.

The culmination of this extremely productive period occurred at the group-theoretical colloquium at Göttingen in June 1939, the central feature of which was a series of lectures by Hall, afterwards published

in *Crelle's Journal* in 1940. His expositions of a classification theory for prime-power groups and of a structure theory for soluble groups, and his papers on automorphism groups and on verbal and marginal subgroups, have taken their place in the canon of essential material for any student of finite groups, and together embrace many of the leading themes of his work up to that date.

Hall's election into the Saldeirian chair of pure mathematics at Cambridge in 1953 marked the beginning of another sequence of publications of the greatest importance. In the intervening years much work had been done—notably by Russian mathematicians—on extensions and generalizations of Hall's theorems on soluble groups, and in 1956 Hall gave a systematic account of these and earlier results on the existence, conjugacy and inclusion properties of subgroups with coprime orders and indices, adding many new results of his own. Another remarkable work on finite groups, carried out in collaboration with Graham Higman, dealt with p-soluble groups and had as its most striking application certain reduction theorems for a celebrated conjecture of Burnside's.

Hall's researches have thrown much light on the interrelation of various finiteness conditions which may be imposed on groups of infinite order, and they have been illustrated by many examples constructed to delimit the range of applicability of the results. Among other types of infinite group considered by Hall may be mentioned Abelian-by-nilpotent groups, locally finite groups and periodic FC-groups.

Hall's influence on British algebraists—of whom he is the acknowledged doyen—has not been confined to specialists in the branch of algebra with which Hall's name, like that of his forerunner Burnside, will be enduringly linked.

Hughes Medal: Prof. A. H. Cottrell, F.R.S.

Prof. Cottrell's contributions to the field of structural metallurgy have been outstanding whether they be regarded from the point of view of the physicist, the metallurgist, or the engineer. Utilizing to the full the current methods of the physics of metals, and in particular the pioneer work of Taylor, Mott and Frank on dislocations, Cottrell has been able to explain, in a remarkably clear physical manner, many important mechanical properties of metals. Typical of this work is his early explanation of the yield-point phenomena in steels, which he showed to be associated with the anchoring of dislocations by solute atoms. He has become a leader in the understanding of the many and decisive ways in which crystal defects, particularly dislocations and vacant atomic sites, control the behaviour of metals in practice.

Engineers and physicists alike owe much to Cottrell's books on *Dislocations in Crystals* and on *Theoretical Structural Metallurgy*. Remarkable for their clarity of thought and composition, they have been a major cause of the current trend towards a more fundamental understanding of their materials by both engineers and metallurgists. In the past decade, British metallurgy has found much inspiration in the work of Cottrell and his associates.

In recent years Cottrell has given attention to irradiation problems arising in nuclear power developments. He has applied his ideas to the effects of irradiation on the properties of both metals and non-metals, and his name is particularly associated with irradiation-induced creep in anisotropic materials such as uranium; but much more than this has been done. It is fair to say that the effects of irradiation on the growth, swelling and creep of uranium, on precipitation in alloys, brittle fracture of steel, and energy storage in graphite have now a solid theoretical background which is largely due to Cottrell.

Leverhulme Tercentenary Medal: Sir Cyril Hinshelwood, F.R.S.

This is the first occasion on which the Leverhulme Medal has been awarded. It will therefore be appropriate to remind Fellows of the circumstances in which it was instituted. In 1960 the Council of the Society accepted an offer from the Trustees of the Leverhulme Trust Fund of a gold medal to mark the tercentenary of the Society. The medal has been named the Leverhulme Tercentenary Medal and is to be awarded every three years to the individual who, in the opinion of the Council, has made the most significant contribution in the field of pure or applied chemistry or engineering. The first Leverhulme Tercentenary Medal has been awarded to our immediate past president, Sir Cyril Hinshelwood.

Few working scientists to-day have not profited from a study of one or more of the profound and far-ranging contributions which Sir Cyril Hinshelwood has made to the advancement of natural knowledge. The range of his interests and attainments is enormous and he has touched nothing which he has not adorned. His publications illustrate not only the diversity of his learning and culture but also its basic unity.

The Leverhulme Tercentenary Medal is being awarded to him for his contributions to pure and applied chemistry. For several decades he has been recognized as one of the leading physical chemists of the world. His work on chemical kinetics is classical; it has combined simplicity and elegance of experimental approach with far-ranging theoretical implications.

He first clarified the mechanism of reactions in the gaseous phase in terms of molecular collisonal processes and molecular activation, and directed attention to the essential characteristics which arise from chain propagation in thermal systems. This work has since stimulated the application of many other methods to the determination of intermediate reactive species. He has made similar contributions to our understanding of reaction mechanisms in both the liquid and solid phases. By applying the same principles to systems involving bacterial growth he has been able to separate phases in the growth process and to examine the effect of inhibitors and the mechanism of drug resistance, and the effect of varying growth media on the growth process. He has investigated the cellular content of nucleic acids during the growth cycle and also the specific effect of elements such as potassium, sodium, phosphorus and sulphur. These researches make common ground between the chemical and biological sciences. Nor has his work been confined to basic science; he has done memorable things in the application of chemistry to useful ends.

He has not only worked as a scientist but, tirelessly, for scientists; for the University of Oxford, for international science, for scientific societies in general and for the Royal Society in particular.

We rejoice that the Leverhulme Medal is being given to a most eminent chemist, and we regard it as particularly happy that a medal which commemorates the tercentenary is going to the man who presided

over that great event with such eloquence, dignity and grace.

ACTIVITIES OF THE ROYAL SOCIETY

It is a pleasure at the end of a year of office to record that I can heartily endorse all that has been said by my predecessors of the admirable way in which the interests of the Society are fostered by Dr. Martin and his staff. The smooth running of its many activities depends to a very large extent on their efforts and I personally am greatly indebted to them for all the help I have received during the past year.

The tasks of my fellow Officers are many and various. We are all very grateful to the Treasurer for the care and attention which he has devoted to the Society's affairs. The Secretaries not only have had to work with their accustomed vigour at their increasingly heavy tasks, but they have had to exercise more than their usual forbearance in dealing with my many naïve questions. I am sure the Society would wish me to thank all these Officers for their work on its behalf.

The Officers and Council always hope that the report of Council has been read by the Fellows, and in any case you will not want me to give you now a catalogue of the Society's activities. There are, however, one or two points which call for comment.

The Society has been so fortunate as to receive a great benefaction from the Wellcome Trust for the establishment of a professorship. We are, indeed, very grateful for this gift, and we are delighted that the name of Sir Henry Dale is to be permanently associated with it, for he has ably served not only the Wellcome Trust but also the Society and, indeed, science generally.

During the past year a number of Fellows have devoted considerable time to surveying the needs of biological research in Britain. Their survey leaves little room for doubt that much more could be done to encourage advances in fundamental knowledge and to foster their application. This may well apply to many branches of physical science as well.

The earning of the national living becomes increasingly difficult in a fiercely competitive world, and it would seem to be self-evident that the country should be straining every nerve to foster science and technology. We must therefore deplore the evident signs that some scientific activities, which to a large extent depend on Government aid, are to be curtailed, or at least are not to be allowed to expand at the present time. Even if this is a 'temporary measure', as it is claimed to be, damage is being done to the confidence of scientists and technologists. They observe that those who control the situation have apparently not yet recognized that the brains of the citizens are one of our greatest remaining national resources, and that the country's future prosperity will be, in a large measure, dictated by the use that is made of this asset.

There are good grounds for believing, for example, that the creation of new senior scientific positions, such as professorships in universities, is not even keeping pace with the expansion in the number of undergraduates. The opinion has been expressed by many Fellows that Britain particularly needs more professorships equipped with adequate research facilities if it is to give reasonable and proper encouragement to all its best men and women. Perhaps we can all do something towards letting it be widely known that we are by no means satisfied with the present overall position.

The maintenance of relations with foreign academies and scientists is one of the important activities of the Royal Society. During the past year this phase of our work has been fully maintained. There have, for example, been visits from Russian and Chinese colleagues, and in September very successful meetings of the Bureau, Executive Committee and General Assembly of the International Council of Scientific Unions were held in London under the sponsorship of the Royal Society. It is very much in the mind of Council to increase as much as possible all activities which impinge even remotely on maintaining contact with colleagues both from the Commonwealth and from other countries. We can only hope that we shall be able to secure means adequate for the task. Not the least of our problems is lack of accommodation for this and other responsibilities that we seek to discharge. Although I cannot give any satisfactory assurances on this point now, strenuous efforts are being made to try to overcome this very serious handicap.

EXCHANGE OF SUBSTANCES BETWEEN THE BLOOD AND TISSUES

On these annual occasions the President has commonly taken the opportunity to make some remarks about matters of scientific interest. This, I suspect, is done mainly to demonstrate that he is not entirely out of touch with scientific experiment. More than once a President has pointed out the impossibility of having a sufficiently wide knowledge to comment adequately on the many outstanding scientific discoveries that are being made under modern conditions. In following in the presidential footsteps, I have been considerably influenced by the remark made by one of my more candid friends, that I was not so highbrow as my predecessors, and by a

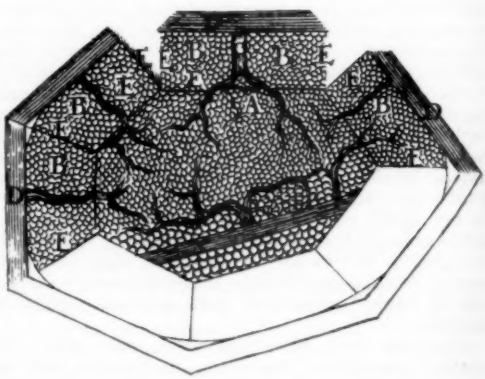

Fig. 1. *Continens Cellulam fimpliciffimam abfque intermediis parietibus aulla in magnitudine.*
A *Cellulæ area interior.*
B *Parietes divulfi & inclinati.*
C *Arteriæ pulmonariæ truncus cum appenfis ramis, quafi reticulato opere definentibus.*
D *Venæ pulmonariæ truncus parietum faftigia pererrans fuis decurrentibus ramis.*
E *Vas in fundo & angulis parietum commune lateralibus, & continuatis retis ramificationibus*

Fig. 1. The first illustration of the capillary circulation with its legend. Malpighi described the capillary circulation in a frog's lung in a letter to Borelli which was published in 1661

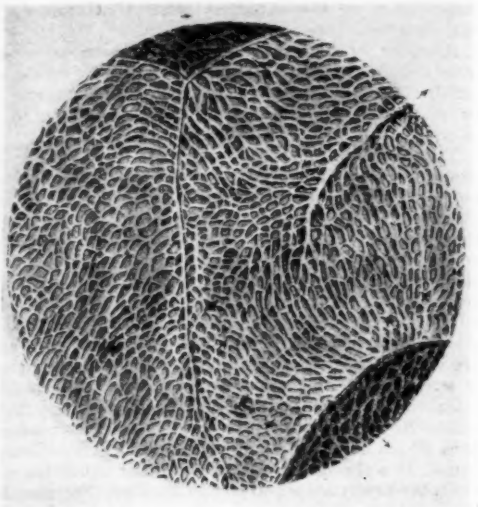

Fig. 2. The capillary circulation in a frog's lung, from a work published by Marshall Hall in 1831 entitled *A critical and experimental Essay on the Circulation of the Blood, especially observed in the minute and capillary vessels of the Batrachia and of Fishes*

comment of another colleague after I had had the honour of delivering a lecture to the Society—he said that it had been a good idea to turn the lights on and off frequently as it kept everyone awake.

Following these hints, therefore, my aim is very modest in that I propose to review and to illustrate briefly some researches in biology the history of which is almost exactly coeval with that of the Royal Society, and which have been greatly influenced by the development of special physical techniques during the past ten years.

In 1628, Harvey published his great work demonstrating the circulation of the blood with irrefutable clarity. The blood left the heart by the arteries and returned to it by the veins. It was not, however, until 1661 that an Italian, who a few years later became a Fellow of the Royal Society and whose portrait now hangs in the Society's house, discovered the vessels which connect the arteries to the veins. I refer, of course, to Marcello Malpighi. By means of his very early compound microscope he observed in the living animal what we now know as blood capillaries.

He saw and illustrated the branching of the pulmonary artery to form a network in the walls of a vesicle in the lung of a frog. The network united to join a pulmonary vein (Fig. 1).

Another Fellow of the Royal Society, Antony van Leeuwenhoek, amply confirmed and greatly extended Malpighi's observations in a large number of species, so that by 1688, in which year Leeuwenhoek sent a letter to the Royal Society describing his observations, there was sufficient evidence to justify the conclusion that blood capillaries were present in all the tissues of animals with a blood circulation.

No great progress was made in the next century and a half in the understanding of the structure of the smallest blood vessels. In 1831, Marshall Hall, a Fellow of the Society who has some claim to have discovered reflex nervous action, wrote: "It is singular that in the midst of the dispute concerning the irritability of the capillaries, there should be a doubt whether the vessels be real tubes or mere canals. There are many reasons which incline me to the latter opinion". That is to say, he thought that they were probably mere spaces in the tissues rather than tubes with their own distinctive wall (Fig. 2).

The knowledge of the structure of the capillary wall was strikingly expanded about the middle of the nineteenth century by the discovery that treatment of the blood vessels, including the capillaries, with dilute silver nitrate resulted in the appearance of a network, which was rightly considered to represent the boundaries between contiguous lining cells. This layer of lining cells, which has proved to be present in all the vessels of the body, was given the name of endothelium (Fig. 3).

As a result of this and other histological observations, the walls of the capillaries were conceived of as being composed of a mosaic of flat cells which were joined together by a cement substance, represented by the material stained black by silver nitrate. In these vessels there are few supporting cells, and the wall is, in effect, composed of endothelial cells with such fibres and tissue matrix as may be closely apposed to them.

It is through the walls of the fine blood vessels that most of the important exchanges of material between the blood and the tissues take place. The walls of the fine blood vessels appear to form a membrane which is freely permeable to gases, such as oxygen and carbon dioxide, which pass to and fro by diffusion. It is thought that substances like sodium chloride and glucose also pass freely, but that the normal capillary wall is almost, though not quite, impermeable to proteins. Ernest Starling, at the end of last century, adumbrated the theory that the capillary wall was of the nature of a semi-permeable membrane, across which the plasma proteins were able to exert an osmotic pressure, and that normally there was a near balance between the hydrostatic pressure of the blood tending to filter fluid out of the vessels and the osmotic pressure of the plasma proteins tending to draw fluid in from the surrounding tissues. This theory of Starling's was given strong support by the experiments of Landis, who measured directly the forces involved, but although Starling's theory is still almost universally accepted it has not been immune from criticism.

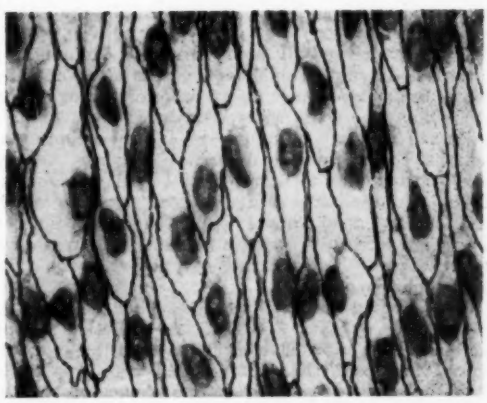

Fig. 3. Endothelium lining the aorta of a rabbit, viewed *en face*. The flat nucleated cells have been outlined by treatment with silver nitrate. (× 390)

Fig. 4. Electron micrograph of a capillary, seen in cross-section, from the subcutaneous tissue of a guinea pig. At this site the wall is composed of parts of two cells; and a nucleus (*n*), mitochondria (*m*), two cell junctions showing adhesion belts (*j*), and many cytoplasmic vesicles can be seen. A basement membrane (*bm*) surrounds the vessel, and external to it collagen fibres (col.) have been cut longitudinally and transversely. (× 12,600.) Inset is a cross-section of a similar capillary viewed with a light microscope with a 2-mm. oil-immersion objective. (× 780)

Much attention has been directed to the detailed structure of the living membrane which was thought to have these semi-permeable qualities. When viewed in cross-section by the light microscope with a 2-mm. oil-immersion lens the capillary wall seems to be composed of a very thin membrane with little recognizable structure except for the nuclei of the endothelial cells, which make a bulge where they occur (inset to Fig. 4.). A basement membrane composed of polysaccharide, and not clearly differentiated from what is known as the pericapillary sheath, is also recognized as a regular constituent of the fine blood vessels.

It was conceived by some that the exchange of water and dissolved solids between the blood and the tissues took place through hypothetical pores in the intercellular cement which, on the basis of the appearances produced by silver impregnation, was thought to be present in substantial amounts. Others believed that minute pores existed in the cytoplasm of the endothelial cells themselves through which substances escaped from the blood. If either of these views were correct, it would not be difficult to visualize conditions in which Starling's hypothesis would be easily credible.

Work during the past ten years has added considerably to our knowledge of the structure of the capillary wall, knowledge which necessitates examining old theories further. This work is a good example of the influence of progress in one science on that in another. Our increased knowledge is due essentially to the invention of the electron microscope, for this remarkable instrument has opened up entirely new possibilities to the biologist who is attempting to correlate structure and function.

Another branch of the physical sciences is also involved in making this possible, for biologists are indebted to polymer chemists for embedding materials in which tissues can be cut sufficiently thin to be examined in the electron microscope.

The electron miscoscope reveals that the membrane forming the wall of a capillary, which is apparently almost structureless and might be likened to a piece of 'Cellophane' when observed in the light microscope, is composed of cells with all the usual cellular organelles (Fig. 4). As well as the nucleus there are small mitochondria, a Golgi complex and a centrosome. There are granules of ribonucleoprotein which are sometimes free and sometimes attached to vesicular structures. The junctions between the cells are regular narrow gaps about 200 Å. wide, and there is no sign of the apparently substantial band of intercellular cement substance which was thought to exist. At the cell junctions there is an electron-dense area which is known as an attachment belt or desmosome. It is thought to be the region at which the cells are most firmly attached to one another. Surrounding the vessel is a basement membrane which apparently contains very fine fibrils.

Palade, the first observer of capillary structure by the electron microscope, described intracellular vesicles which, though found in other cells, are particularly prominent in endothelium. On the luminal surface of the endothelial cell there are indentations of the bounding membrane known as caveolæ intracellulares. Similar caveolæ exist on the external surface of the cell. Between these, two surface profiles of vesicles, which are usually some 500–600 Å. in diameter, can be recognized in the cytoplasm of the cell (Fig. 5).

Palade proposed the theory that fluid is transported from the lumen of the capillary to the outside by means of these structures. According to this theory, a minute amount of plasma is enclosed in the caveola, which is then pinched off by a membrane to form an intracytoplasmic vesicle. The vesicle is ferried across the cytoplasm to be discharged on the external surface beneath the basement membrane. It is difficult to see how the cells of the capillary wall can exercise

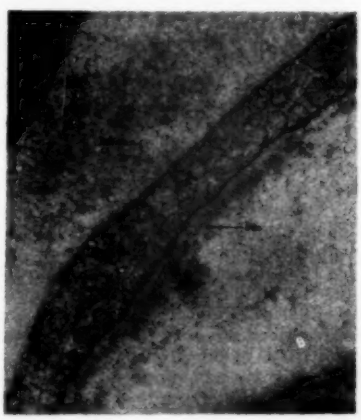

Fig. 5. Capillary wall from a normal rat heart fixed immediately after death. Part of the cytoplasm of an endothelial cell is seen, showing caveolæ intracellulares and vesicles. The basement membrane appears as a dark band (*bm*) along the external surface of the cell. (× 28,000)

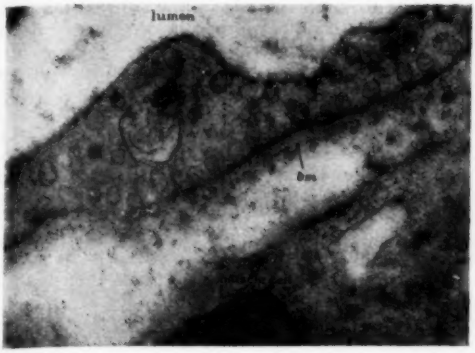

Fig. 6. Capillary from a rat heart perfused for 15 min. with saline solution containing particles of saccharated iron oxide. Particles are present in the lumen of the vessel and in the pericapillary space. They can be seen in the caveolae intracellulares on both the luminal and external surfaces of the cell, and in vesicles in the cell cytoplasm. *bm*, basement membrane. (× 33,600)

selective permeability if this is the mechanism for fluid transport, since the vesicles are very much bigger than protein molecules. To meet this difficulty Palade proposed that the selective permeability of capillaries resided in the basement membrane, which is usually a well-marked feature in the capillary wall.

Palade and Wissig attempted to show that the vesicles did, in fact, constitute a transport mechanism by injecting into the blood stream the protein ferritin, the large molecule of which contains sufficient iron to make it opaque to electrons. They found that ferritin molecules could be seen in some of the vesicles but that they also lay free in the cytoplasm. Later Palade used colloidal gold in the same way, and stated that the particles of colloidal gold were always found within the vesicles and not free in the cytoplasm, and he inferred that fluid must be transported through the endothelial cells in the vesicular system.

One of the difficulties attending such observations is to procure a sufficient concentration of the electron-opaque particles to act as efficient markers, but this can be overcome to a certain extent by the use of perfused organs. It is a standard pharmacological technique to make an isolated rat or rabbit heart beat for hours by perfusing it with a suitable oxygenated saline solution. It is possible, without stopping the heart beat, to add to the perfusion fluid a considerable amount of colloidal thorium dioxide or colloidal ferric hydroxide, the particles of which are, of course, opaque to electrons. Using such a preparation, Jennings and Marchesi have shown that the particles of ferric hydroxide pass into the open mouths of the caveolæ intracellulares on the luminal surface of the endothelium, and that they appear in the vesicles, in the cell cytoplasm, and in the caveolæ which open at the external surface. It seems fairly certain that these particles are in passage across the cell in the vesicular system in the manner proposed by Palade. There is not much evidence that the particles pass between the cells at the intercellular junctions. After they have passed through the cell there is no evidence from electron micrographs that the basement membrane forms much of a barrier to these particles, so that it is difficult to conceive that it would do so to molecules of protein (Figs. 6 and 7).

One may, therefore, picture the formation of caveolæ and vesicles as a process which is continuously altering the capillary wall and passing fluid from the lumen through into the tissue spaces. It is probable that the caveolæ are too large to account for the semi-permeable qualities of the endothelial membrane. The electron microscope, although its resolving power is sufficient to do so, has not revealed any smaller channels passing from the lumen to the outside of the capillary. Such channels would be necessary in a membrane which was semi-permeable by reason of the size of the pores in its structure. It still remains, therefore, to be explained in what structure or mechanism the semi-permeability of the normal capillary wall resides.

A morphological discovery entirely due to the electron microscope is that the capillaries do not have the same structure in all parts of the body. Those in the glomerulus of the kidney have small fenestrations through the cell cytoplasm, and those in the endocrine organs such as the pituitary body, the adrenals and the thyroid gland have gaps in the cytoplasm which are filled by a very tenuous membrane. Similar capillaries are found in the small and large intestines. There is no evidence, except in the case of the kidney, that these gaps are particularly permeable to proteins and their significance is not yet understood.

During the past forty years it has been demonstrated that certain substances which occur in the tissues of animals, such as histamine, 5-hydroxytryptamine, and bradykinin, have the property, when they are applied at great dilution, of making blood vessels more permeable than normal to proteins. Swelling or œdema is an important feature in many natural inflammations, such as those produced by bacterial invasion, and it is possible that substances of this nature which seem to be liberated in injured tissues are responsible for the increased permeability of the capillaries to proteins, leading to the accumulation of extravascular fluid. Majno and Palade have recently examined the small blood vessels in the cremaster muscle of the rat after it had been subjected to the influence of dilute solutions of the vasoactive substances that I have mentioned. They could find no

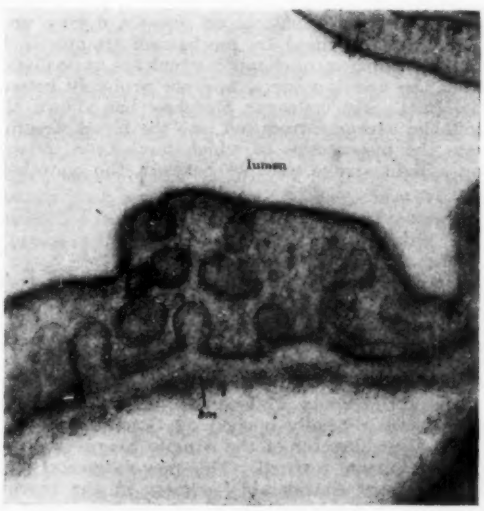

Fig. 7. From the same heart as Fig. 6. Particles of saccharated iron oxide are present in the luminal caveolae and in three cytoplasmic vesicles which are apparently joining together. *bm*, Basement membrane. (× 75,000)

Fig. 8. Venule in the mesentery of a rat. A suspension of carbon was injected intravenously 1 min. after the mesentery had been inflamed by mechanical injury, and the tissue was fixed 3 min. later. Carbon particles have passed into a space in the vessel wall which may well be at a junction between two endothelial cells. *bm*, Basement membrane; *e*, endothelium; *pe*, periendothelial cell. (× 24,600)

alteration in the vesicular structures of the endothelium, and no holes through the endothelial cytoplasm itself, but there were discontinuities in the walls of some of the minute vessels which were probably at the junctions between the endothelial cells. These discontinuities let through not only plasma and fine particles of mercuric sulphide but also the much larger blood platelets. The plasma clotted outside the endothelial cells with the formation of fibrin, and the particles and the blood platelets were held up by the basement membrane.

In a similar way it appears that the increased permeability of small blood vessels during acute inflammation caused by mechanical trauma is due to the opening up of channels which are quite distinct from the vesicle system, and are probably between the cells. My colleague Marchesi has shown that colloidal carbon introduced into the blood stream of rats the mesenteries of which have been inflamed by a light stroke, escapes between the endothelial cells of the minute vessels in the injured area, and becomes for a time entrapped between the cells and the periendothelial tissues (Fig. 8).

Thus there is evidence to suggest that in normal vessels transport of fluid may be principally through the substance of the endothelial cells, but that in pathological conditions there may be an actual solution of continuity of the vessel wall, probably at the cell junctions, enabling much more fluid, as well as solid bodies, to pass through. This solution of continuity may be of a very labile nature, that is to say, the endothelial cells may part and join together again rapidly, but the overall effect is to allow a considerable leakage of plasma from the inside to the outside of the vessels.

The researches of the past nine or ten years have considerably increased our knowledge of the membrane through which exchanges of essential substances take place in those animals with a circulatory system. We are still far from understanding the precise behaviour of this complicated structure even under the simplest physiological conditions. What is clear is that mathematical concepts for explaining the transfer of water and large molecules through the walls of small blood vessels on mechanical principles will have to take cognisance of what is now known of their structure. The conceptions arrived at from the morphological and mathematical approaches have not yet been reconciled. Indeed, one may wonder whether the long-discarded view of Heidenhain, that lymph is secreted by the walls of the capillaries, may not have a grain of truth in it, for if the vesicle transport theory should be supported by further evidence, it may become necessary to postulate that work is done during the transport of material across the vessel walls, and this could be construed as secretion.

It is perhaps astonishing that we are still so unsure of the mechanism of the exchange of substances between the blood and tissues for, as Marshall Hall pointed out, the whole elaborate apparatus of heart and large blood vessels exists merely to subserve this exchange which takes place in the finest vessels.

Science is rarely advanced by what is known in current jargon as a 'break-through'. Rather does our increasing knowledge depend on the activity of thousands of our colleagues throughout the world who add small points to what will eventually become a splendid picture, much in the way that the Pointillistes built up their extremely beautiful canvases. What I have mentioned here are a few such points which it has taken three hundred years to place in the picture.

UNIVERSITY COLLEGE OF NORTH STAFFORDSHIRE

EXTENSION TO DEPARTMENT OF PHYSICS

THE new extension of the Physics Department of the University College of North Staffordshire, Keele, was officially opened on October 5, by Dr. F. A. Vick, director of the Atomic Energy Research Establishment, Harwell. This new extension represents a very considerable increase in the teaching facilities at Keele and the extra floor area that has been added to the Department amounts to some 18,000 sq. ft. Most of the space in this new extension is actually devoted to undergraduate and post-graduate teaching in the form of either lecture theatres, tutorial and colloquium rooms, or large teaching laboratories. Since the space that had been used in the old building for teaching has now been liberated for expansion of the Department's research activities, an increase for the latter has also been made possible, by the completion of this building.

The new building has a somewhat striking appearance (Fig. 1), and the main frontage is formed from vertical buttresses which are finished in large grey

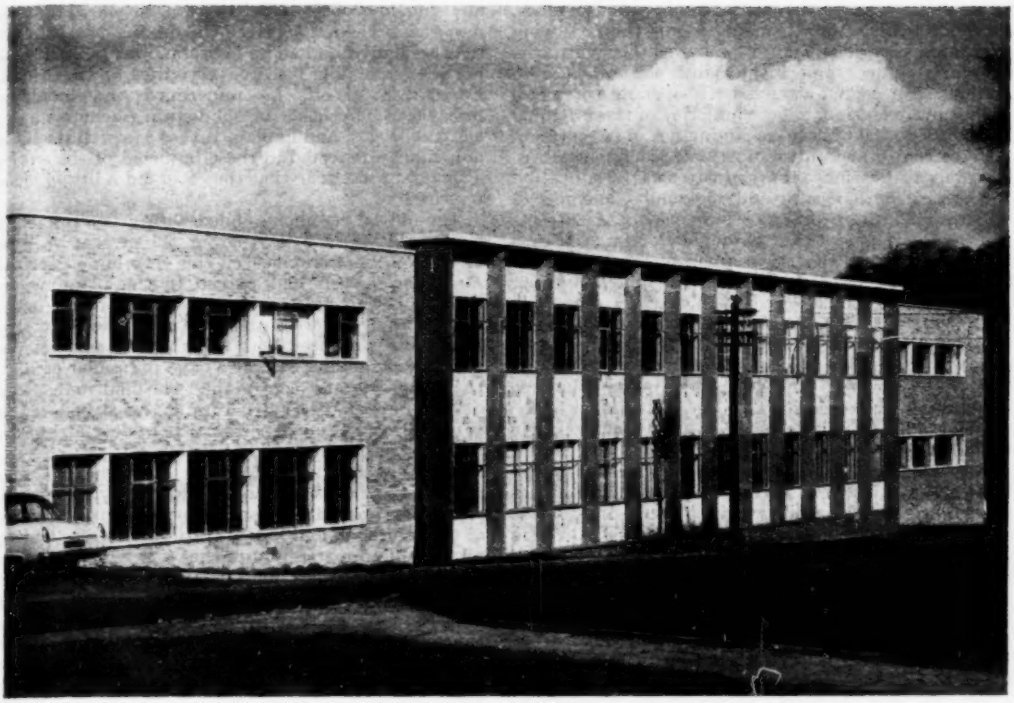

Fig. 1. New extension of the Physics Department, University College of North Staffordshire

slate slabs with inset walls of sparkling quartzite. The contrasting contours and colours provided by this southern frontage give a very pleasing impression when viewed in autumn sunlight. The building has two main floors, the ground floor being on the same level as the single-storey building which has previously housed the whole of the Department, and connects with it through a wide corridor with swing doors intervening. One somewhat unusual feature of the actual construction was the necessity of building the whole structure in five separate portions so as to meet the anti-subsidence conditions which affect the site. It is, in fact, extremely difficult to recognize these separate sections now that the building has been completed and an air of integrated cohesion exists throughout the Department, new and old buildings included. The central feature of the new wing is the large lecture theatre which is situated in the middle of the structure and continues through both floors. It has been designed not only to meet the needs of the expanding numbers in the physics courses themselves but also for the time being to house the whole of Keele's foundation year consisting of about 230 students. Corridor space has therefore been provided around the whole of this lecture theatre together with a large open entrance hall stretching up to the roof of the building itself. This has already proved of very great value as an assembly and discussion area for the large number of students that are now using the building. One feature of this hall which has produced considerable comment has been the overhead lighting, which consists of fluorescent tubes mounted within a covered glass structure which is opened to the sky through the roof. The resulting effect is almost identical during the day or night, since in daytime the sunlight itself is diffused through the whole structure over the entrance hall, while at night the fluorescent light is diffused in a very similar way and a very similar effect is obtained.

There are also two smaller lecture theatres as well as the main theatre, one which will hold up to 60 students and the other about 40. Together with these is a series of other teaching rooms, including a colloquium room for postgraduate seminars, a reading room for use by both undergraduate and research students, and a tutorial room for the tutorials which Keele still hopes to maintain as an essential part of its teaching system. The teaching laboratory accommodation consists of three large laboratories and one rather small optics laboratory serving the first honours year, since specialized teaching is still being carried out in the old building, including the teaching of optics in the second and third honours years. Two of the main teaching laboratories are arranged one above the other and are identical in size and design, each being 44 ft. long and 30 ft. wide, and are designed to provide sufficient accommodation for experimental work by up to 50 students. Each laboratory has a technician's service room leading from it, which contains all the rectifier and control equipment. The services in the teaching laboratories have been carefully kept around the walls and also fed to island frames in the centre of the room. The benches on which the experiments are performed are thus entirely flexible, and their location and assembly can be adjusted to the needs of the particular experiments that are being carried out. Electrical supplies are carried in continuous trunking with four standard types of outlet panels providing either 3-phase a.c.,

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single-phase a.c., or d.c. voltages of various values. As well as these main teaching rooms the new wing also houses various staff rooms together with the secretarial offices and technicians' accommodation.

About 250 people attended the opening ceremony of the new extension, a large majority of these coming from outside the College, including not only representatives from other universities and teaching institutions but also various representatives of the education authorities, both locally and regionally, and also a large number from different industries and Government research stations throughout the country. Sir Alfred Owen, who is chairman of the University College Council, took the chair at the official opening ceremonies, and first called upon the new principal, Dr. H. M. Taylor, to introduce the guest of honour, Dr. F. A. Vick. In introducing Dr. Vick, Dr. Taylor referred to the rather incongruous position in which he found himself as introducing a speaker who was probably much better known to a large number of the audience than he had been to Dr. Taylor himself. He explained, however, that Dr. Vick's enthusiasm and interest in the more basic and liberal approach to the teaching of a scientist had already made its impact on Cambridge during his own time there, and he was thus already very much acquainted with Dr. Vick's thoughts and ideas if not so directly with Dr. Vick himself. The audience was indeed very fortunate in having as its main speaker not only the director of the Atomic Energy Research Establishment but also the ex-professor of physics at Keele himself.

In his own speech Dr. Vick outlined the history of the Physics Department during its eleven years existence, starting from the single room in Keele Hall and explaining that the first extension of the Department had consisted of the acquisition of two cupboards and half a room in the basement. The first main stages of the Physics Department building itself were built in 1951 and 1952, and Dr. Vick explained that they had been purposely designed for research uses as much as for teaching with the idea that the main teaching block should follow later. It was in fact this block itself that was now being opened, thus completing the steps in the original plan. Dr. Vick's historical summary was lightly sprinkled with amusing anecdotes which served to show that the major experiment at Keele in mixing the arts and sciences had often had its lighter moments. In discussing the expanding research activities of the Physics Department itself, he underlined the increasing interest of physicists in solid-state phenomena and gas discharges, and explained that Harwell itself was now much interested in these branches of the subject although it was true that they still did a little nuclear physics!

The official ceremony ended with a vote of thanks given by Prof. D. J. E. Ingram, the present professor of physics, who emphasized the very large part that Dr. Vick himself had played in the planning and design of the new physics building and said he felt sure that all who were present would agree that these efforts had produced both a highly efficient yet very handsome building. He also gave particular credit to the architects and contractors who had managed to design and build the structure at a cost of less than £100,000, which must be somewhere near a record in these days of rising building prices.

The new buildings, together with the old wing, which is now devoted almost exclusively to research, were then opened for general inspection for the rest of the afternoon and it was gratifying to see the large number of animated groups that sprang up discussing both research topics and laboratory design. Some of these design features arose from the particular circumstances at Keele, such as the anti-subsidence foundations and the heating which is entirely by inset panel radiators fed from the College boiler house. One other characteristic feature of the building was the large projection room behind the main theatre housing the ciné projector and other lantern equipment. This is a particularly useful feature for the foundation year lectures, when all types of teaching methods are being used by the different departments throughout the College.

One other very fortunate feature of the opening ceremony was that it had proved possible to bring the new building into operation just before the new academic session started, and all was in fact ready for the lectures and classes which were due to begin two days later.

D. J. E. Ingram

FUR SEALS BREEDING IN THE FALKLAND ISLANDS DEPENDENCIES

By F. A. O'GORMAN

Falkland Islands Dependencies Survey, 4 Millbank, London, S.W.1

BETWEEN December 1957 and March 1958 a Falkland Islands Dependencies Survey party of geologists and surveyors visited Livingston Island in the South Shetlands. On January 14 twenty-seven fur seals (*Arctocephalus tropicalis* (Gray)) on Cape Shirreff on the north coast were found, and on February 1 a further fifteen near Start Point in the north-west corner. There was no evidence that breeding had occurred[1].

The following year a party, led by me, was organized to carry out a preliminary investigation of the South Shetland fur seals, and during this visit a breeding group was found on Cape Shirreff on January 30. On February 29, 1960, H.M.S. *Protector* visited the South Sandwich Islands and found breeding fur seals on the south-east coast of Visokoi Island[2,3].

These are the first records of fur seals in any of these islands since 1902 when the Swedish Antarctic Expedition found one animal on Nelson Island in the South Shetlands[4], and the first record of breeding since the last century.

The Falkland Islands Dependencies consist of the sector lying south of South America between longitudes 20° and 80° west, and include the Graham Land Peninsula and the Islands of South Georgia, South Sandwich, South Orkneys and South Shetlands. South Georgia and South Shetlands saw the greatest part of the fur-sealing operations of the early eighteenth century. The present status of the fur seal on South Georgia has been discussed by Bonner[5].

The discovery of the South Shetlands in 1819 by Smith was closely followed by intensive sealing.

James Weddell[6] in 1825 reported that, between 1820 and 1822, 320,000 fur seals were killed and the colonies were virtually exterminated. At Cape Shirreff alone, 95,000 seal skins were taken in the season of 1821 by five British crews[7]. More than ninety ships were engaged in sealing around the South Shetlands during this period[8].

After this period of abundance, small catches were obtained up to 1871 when the re-developing colonies were again devastated. Between 1871 and 1890 more than 35,000 seal skins were taken[8,9]. The last definite report of the fur seal was on Nelson Island, though Hamilton[10] gives an unsubstantiated report of Canadian sealers taking animals up to 1904. Between 1925 and 1951 *Discovery*, *Discovery II* and *William Scoresby*, the ships of the *Discovery* Committee, made extensive voyages in the Dependencies and visited the South Shetlands, the South Orkneys and South Sandwich Islands without finding any fur seals[11–15]. A small number of fur seals were, however, found on South Georgia[16,17].

Fig. 1. Fur-seal family on Livingston Island, South Shetland Islands, February 2, 1959

In 1959 the Cape Shirreff colony, obviously at its earliest stage, consisted of one adult female and her male pup and seven adult males. A dead pup, also male, was found nearby. The seals were hauled out on a small windswept gravel beach frequented by groups of moulting elephant seals (*Mirounga leonina*), small numbers of Weddell seals (*Leptonychotes weddelli*) and numerous chinstrap (*Pygoscelis antarctica*) and gentoo penguins (*P. papua*), none of which had any apparent effect on the behaviour of the fur-seal group. The colony was watched intensively during January 31–February 4. On February 3 a second female, possibly the mother of the dead pup, arrived; no further change in the size of the colony occurred during this period. Some harem maintenance was being carried out by the dominant bull, but not very energetically. Quite often the female sparred and played with one or two of the younger males present, sometimes chasing each other in and out of the sea. The live pup measured 2 ft. 4 in. (71·1 cm.) in length and weighed approximately 15 lb. (6·8 kgm.) which, when compared with figures on the growth-rate in *Arctocephalus tropicalis* in South Georgia[18], would suggest that the pup was born about January 27. The pup was tagged with a monel metal cattle ear tag in the axilla of the right fore-flipper. On March 20 thirty-two fur seals were counted in the vicinity.

Unfortunately, it has not been possible to procure any further information on the subsequent development of this group, despite its considerable biological interest as one of the few mammal colonies where the natural growth could be studied from its inception.

The species has been identified, from a skull and skeleton, by Miss J. King of the British Museum (Natural History), as *Arctocephalus tropicalis gazella* (Peters, 1875); the same as that which occurs in South Georgia[19,20] and the South Orkneys.

The South Sandwich Islands, the most eastern islands of the Scotia Arc have never been renowned for fur seals. There are apparently no records of them having been laid waste in the 1820's as were those of the South Shetlands. Morrell, whose account of his voyages is generally discredited[21], found none in 1822, but Captain Buddington[9] states that in the 1875–76 season 2,000 were taken at the northernmost island (Zavodovski) and that, in 1876–77, 4,000 were taken by six vessels. In the following year the catch did not exceed 100. In 1880–81 no seals were found and in 1891–92 400 were obtained and a further 200 seen. Larsen visited several of the islands in 1908 but failed to find any[11]. These appear to be the only records of fur seals until the visit in 1960 of the Governor of the Falkland Islands, Sir Edwin Arrowsmith, on board H.M.S. *Protector* (Captain Forbes, R.N.). The only group of fur seals was found at the south-east corner of Visokoi Island on a beach which consisted of volcanic gravel and isolated outcrops of rock over which the seals sprawled. Sir Edwin Arrowsmith and Captain Forbes estimated the numbers to be in the region of 400. Photographs of the colony show a number of harem bulls and a loosely knit harem system. Several black pups can also be seen. None of the other islands in the group was visited. However, during January 7–11, 1961, the Falkland Islands Dependencies Survey vessel R.R.S. *Shackleton* visited the group to carry out geomagnetic observations, but only one fur seal was found on Visokoi Island and none was seen on the Islands of Zavodovski, Leskov, Candlemas, Vindication or Saunders. It must be emphasized that no special effort was made to locate fur seals. Though there were no skins or skulls collected from the South Sandwich fur seals, from their appearance they would seem to be similar to those of the South Shetland and South Georgia and are presumably the same species. Fur seals were also found on Clerke Rocks (55° 01′ S., 34° 41′ W.) on January 6 [22,23].

The discovery of these colonies and of the one on Powell Island in the South Orkneys in 1960 [24] demonstrates the rapid recolonization of this section of the Antarctic by the fur seals, with South Georgia presumably acting as the reservoir for this expansion.

Although no positive anatomical proof is available, it is reasonable to suppose that the fur seals of the South Shetland and the South Sandwich Islands (and

South Orkneys) present in the 1820's were also *Arctocephalus tropicalis*, and not *A. australis* as has been assumed until quite recently. Poland[25], in 1892, seems to have been in little doubt as to the differences between the species of fur seal on the Falkland Islands and the mainland of South America and those on South Georgia, South Sandwich and South Shetland Islands, his conclusions being based on the characteristics of the pelt. A fur seal upper canine tooth dug up from among remains of an early nineteenth century sealing camp on Livingston Island is not, unfortunately, referable to either species, but a more detailed search at this site might reveal material specifically identifiable.

It is hoped that these colonies, especially those in the South Shetlands will be strictly protected in keeping with the resolution on conservation in Antarctica passed by the Special Committee on Antarctic Research of the International Council of Scientific Unions[26], and allowed to re-inhabit the areas where they were once so numerous and so wantonly destroyed.

I wish to thank Sir Vivian Fuchs for permission to publish this article and Sir Edwin Arrowsmith and Captain David Forbes for the photograph and information on the South Sandwich Islands.

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OBITUARIES

Mr. M. A. C. Hinton, F.R.S.

With the death on October 3 of Mr. Martin A. C. Hinton there passed one of the last great amateur naturalists of the Victorian era, whose contributions to geology and zoology earned him world-wide renown.

Martin Hinton was born in London on June 29, 1883, the eldest of three children. His formal education ended at the age of twelve when he left school to begin legal work in the City. His interests in natural history were already growing and he was a frequent visitor to the Museum of Practical Geology, then in Jermyn Street, where handling the collections and talking with the curators he rapidly acquired an extensive knowledge of the subject. After eleven years as a 'voluntary worker' at the British Museum (Natural History), he joined the staff as an assistant in the Zoological Department in 1921; he was made deputy keeper in 1927 and keeper in 1936, a post which he held until his retirement in 1945.

In 1899, when only sixteen years old, he read his first paper to the Geologists' Association on the Pleistocene deposits around Ilford and Wanstead. This was the beginning of a series of papers on the Pleistocene geology of the Thames valley which continued for a quarter of a century. He did pioneer work in recording sections in the Thames gravels, he collected and identified the fauna they contained and gave stimulating if sometimes unorthodox interpretations of the Pleistocene history. His views are summed up in a paper read to the Yorkshire Geological Society in 1926.

On joining the staff of the British Museum (Natural History), Hinton directed his attention to faunal studies, especially of rodents, in which group he made major contributions. His painstaking work on these small and difficult mammals includes taxonomic investigations on voles and lemmings, British fossil rodents and lagomorphs; he was early to see the potential use of rodents for stratigraphical correlation of Pleistocene deposits, a technique which has become increasingly valuable. But he was not to be limited to the dry bones of the Museum collections; he took a keen naturalist's interest in the living rodents, and his British Museum booklet on *Rats and Mice as the Enemies of Mankind* stresses cogently the immense destructive potential of these pests. In the 1930's Hinton was largely responsible for the success of the Ministry of Agriculture's campaign to exterminate the muskrat in Britain, which had at that time become a major hazard, breeding very rapidly and undermining river and canal banks and even gravel railway embankments.

At the opposite end of the size-range in mammals, Hinton devoted his attention to whales, contributing reports on antarctic whales for the Colonial Office and collecting a school of false-killer whales stranded on the Dornoch Firth; he clearly appreciated the importance of studying populations as opposed to individuals, and his salvage of 127 whales from a remote part of Scotland and transporting them to London was a triumph of organization and perseverance.

Hinton carried out a large measure of administrative duties both within the British Museum and in various learned societies; for some years he was an editor of the *Zoological Record*, and zoological secretary of the Linnean Society; he served on the councils and was a vice-president of both the Zoological Society and the Linnean Society. He was elected to fellowship of the Royal Society in 1934.

Hinton was by nature shy, but in the company of friends he was unsurpassed as a raconteur. He was blessed with robust health throughout life, and

possessed a remarkably retentive memory; failing eyesight in later years virtually prevented him continuing with scientific pursuits. His first wife, by whom he had three children, predeceased him, and shortly after his retirement he married Dr. Dina Dobson and went to live in Somerset close to the Mendip Hills, where his early interests in Pleistocene cave faunas had grown. He rarely went to London in later years, but all who visited him, professional or amateur, novice or doyen, were assured of a very warm reception, and the benefit of his sage advice.

R. J. G. Savage

Dr. G. H. Young

Dr. George H. Young, whose sudden death on October 10, 1961, at the age of fifty-two is reported from Pittsburgh, was a chemist known principally in the United States for his work in the field of organic polymers, resinous coatings, corrosion protection and industrial and marine paints.

During the Second World War he was a specialist consultant on corrosion and protective coatings to the U.S. War and Navy Departments, as a result of which he became known internationally for some joint studies with Drs. Seagren and Zehner on the interaction with steel of anti-fouling paints and he received the U.S. Navy's Bureau of Ordnance Development Award in 1945.

He served as secretary to the technical committees which initiated the war-time synthetic rubber programme and was continuously associated informally with this major U.S. Government undertaking until its termination in 1955. He had been a special consultant to the Department of Defense and was a member of the National Research Council's Material Advisory Board. He also served as technical consultant to the Pennsylvania General State Authority.

Since 1951 Dr. Young had been a director of research at the Mellon Institute, where he had been active in many spheres of administration and consultation, as well as patent adviser to the Institute's scientific personnel, having been admitted to practice before the United States Patent Office in 1940.

To his many activities Dr. Young added membership of numerous scientific and technological societies, including some in Britain and Germany, and in 1949 he served as U.S. delegate to the United Nations Conference on the Conservation and Development of Natural Resources.

O. D. Hunt

Prof. W. N. Bailey

Prof. Wilfrid Norman Bailey, who died at Eastbourne on October 23, was born at Consett, County Durham, on September 5, 1893. He soon displayed his mathematical abilities, and after completing a brilliant undergraduate career at Trinity College, Cambridge, becoming a Wrangler in 1915, he resigned a Research Exhibition to take up war service. During 1915–19 he was an instructor lieutenant in the Royal Navy in which capacity he witnessed the Battle of Jutland. In later years he looked back on this period of his life with a certain nostalgia. When war service was finished, he held a number of university appointments at Birmingham and Manchester, and in 1944 he was appointed to the chair of mathematics at Bedford College, University of London, from which he retired in 1958.

Bailey's interest in mathematics was focused on the theory of hypergeometric series and Bessel functions, and on these topics he published some fifty papers in addition to the well-known Cambridge Tract which he wrote on *The Hypergeometric Series*. During the whole of his life these subjects retained their fascination for him and he had, to an exceptional degree, the capacity for conveying his excitement to others. He was normally of rather a placid demeanour, but it was a revelation to hear him lecture on any topic which had aroused his interest.

Prof. Bailey took a full part in the mathematical life of the country, and was at various times librarian, editor, secretary and vice-president of the London Mathematical Society. Although he found committee membership somewhat tedious, he always accepted any work of this nature which he felt it was his duty to undertake.

Prof. Bailey is survived by a widow, a son and a daughter. He will be remembered by those who knew him both for his intense enthusiasm for mathematics and for his genial sociability.

H. G. Eggleston

Dr. Marian W. Smith

Dr. Marian W. Smith (Mrs. H. F. Akehurst), honorary secretary of the Royal Anthropological Institute since 1956, died on May 2, after a long illness.

Marian Wesley Smith was born on May 10, 1907, in New York, and gained her master's degree at Columbia University in 1934, proceeding to the doctorate in 1938. Between 1937 and 1947 she taught in a number of American universities, and held office in several learned societies, including the presidency of the American Ethnographic Society during 1944–45. During this period too she carried out extensive field work on the Indians of Washington State and British Columbia, resulting in monographs of the Pyallup-Nisqually (1940) and the archæology of the Columbia-Fraser River (1950) as well as a number of papers in learned journals. She later did work in Pakistan, but owing to her untimely death this was never brought to the stage of publication.

She was already known as a leading anthropologist, notable for the breadth of her view, when, in 1952, she married an Englishman, Mr. H. Farrant Akehurst, and made her home in Great Britain. She undertook part-time teaching work at the London School of Economics, and in 1956 she accepted the honorary secretaryship of the Royal Anthropological Institute. It was this part of her work which made her known to and loved by practically all British anthropologists. She devoted a great deal of time to the affairs of the Institute, including a much-needed overhaul of its finances, which was carried out in close collaboration with the honorary treasurer, Sir George Beresford-Stooke, and helped by the magnificent generosity of Mrs. Brenda Seligman. She initiated an excellent tradition by organizing a succession of symposia on subjects marginal to anthropology, namely, race problems, primitive music and the domestication of cattle, thereby making use of the specialized knowledge of many experts in other fields of investigation.

Her ability, her devotion to the Institute, and above all the warmth of her personality were such that every regular attendant at the meetings of the Institute will feel the loss of a personal friend.

A. E. Mourant

XUM

NEWS and VIEWS

The Royal Society: Officers for 1962

At the anniversary meeting of the Royal Society on November 30, Sir Howard Florey, professor of pathology in the University of Oxford, was re-elected president. The other officers re-elected for the ensuing year were: Treasurer, Lord Fleck, formerly chairman of Imperial Chemical Industries, Ltd.; Biological Secretary, Sir Lindor Brown, Waynflete professor of physiology in the University of Oxford; Physical Secretary, Sir William Hodge, Lowndean professor of astronomy and geometry in the University of Cambridge; and Foreign Secretary, Sir Patrick Linstead, rector of the Imperial College of Science and Technology. Other members of Council elected (or re-elected) were: Mr. F. C. Bawden, director of Rothamsted Experimental Station; Mr. E. J. H. Corner, reader in plant taxonomy in the University of Cambridge; Prof. J. E. Harris, professor of zoology in the University of Bristol; Sir Harold Himsworth, secretary, Medical Research Council, consulting physician to University College Hospital, London; Prof. A. F. Huxley, Jodrell professor of physiology at University College in the University of London; Sir Christopher Ingold, emeritus professor of chemistry at University College in the University of London; Prof. W. T. J. Morgan, deputy director of the Lister Institute and professor of biochemistry in the University of London; Prof. C. F. Powell, Melville Wills professor of physics in the University of Bristol; Prof. O. A. Saunders, professor of mechanical engineering at the Imperial College of Science and Technology in the University of London; Dr. C. J. Stubblefield, director of the Geological Survey of Great Britain and the Museum of Practical Geology; Sir Gordon Sutherland, director of the National Physical Laboratory; Sir Graham Sutton, director-general of the Meteorological Office; Dr. H. W. Thompson, reader in infra-red spectroscopy, University of Oxford; Prof. C. H. Waddington, professor of animal genetics in the University of Edinburgh; Prof. A. G. Walker, professor of pure mathematics in the University of Liverpool; Prof. F. C. Williams, professor of electrical engineering in the University of Manchester.

Psychology at Cardiff: Prof. G. Westby

Mr. G. Westby, who has been appointed to be the first holder of the new chair of psychology in the University College of South Wales and Monmouthshire, is at present head of the Department of Psychology at Hull. He is well known by reason of his outstanding success in developing his present department during the past twelve years. Starting almost from scratch, its activities now include a large and flourishing honours school, the high standards of which are widely admired, and a varied and enthusiastically pursued programme of research. The broad basis, coupled with the strong integration, of the Department have enabled it also to make a full contribution to interdisciplinary courses at the honours level and to the training of graduate students in the social sciences.

Mr. Westby, after reading the Honours School of Philosophy, Politics and Economics, acquired considerable experience in the education and treatment of maladjusted children before returning to Oxford after the War to gain a distinction in the diploma in psychology in 1946. Soon after he went to Hull it became apparent that he commanded the wide understanding of issues in the many diverse aspects presented by contemporary psychology, and the power to attract to his staff people of ability and initiative, without which all effort to build a living department must be in vain. These talents will find full scope at Cardiff, where exceptional possibilities exist, not only because of the keenness for a department of psychology among all the faculties concerned, but also by reason of the proximity and interest of the Welsh National School of Medicine. When difficulties inseparable from starting almost at the beginning have been surmounted, and the advantages exploited, we may confidently look forward to the establishment of a notable new centre of psychological studies.

Atlas Computer Laboratory, National Institute for Research in Nuclear Science: Dr. J. Howlett

Dr. J. Howlett has been appointed head of the *Atlas* Computer Laboratory of the National Institute for Research in Nuclear Science. The appointment is effective from December 1. This Laboratory, to be built adjacent to the Institute's Rutherford High Energy Laboratory at Harwell, will house an *Atlas* electronic digital computer. Dr. Howlett, born in 1912, was educated at Stand Grammar School, Manchester, and the University of Manchester, where he read mathematics. Following an initial period in the Research Department of the London, Midland and Scottish Railway Co., Dr. Howlett joined the Ministry of Supply in 1940, where he was engaged in mathematical and computational work on a variety of defence problems. Since 1948 he has been head of the Computing Group of the Theoretical Physics Division at the Atomic Energy Research Establishment, Harwell.

Courtaulds, Ltd.: Appointments

Sir Alan Wilson, a deputy chairman of Courtaulds, Ltd., has been appointed chairman of the company in succession to Sir John Hanbury-Williams, who retires next summer. Sir Alan was elected to the Royal Society in 1942 in recognition of his work on the electronic theory of solids and on the properties of metals, and joined Courtaulds in 1945. Mr. C. F. Kearton and Mr. H. R. Mathys have been appointed deputy chairmen of Courtaulds. Mr. Kearton has been with the firm since 1946, and was elected to the Royal Society in 1961 for his work on the chemical engineering characteristics of volatile uranium products and technological developments in the textile industry. Mr. Mathys is a chartered patent agent and was in general practice until the outbreak of war in 1939; he joined Courtaulds in 1946 to take charge of the Patent Department at Coventry, and was appointed a director in 1954.

Nora: a New Zero Power Reactor in Norway

The zero power reactor, *Nora*, of the Norwegian Atomic Energy Institute (IFA), which will serve in connexion with the joint research programme in reactor physics of the International Atomic Energy Agency (IAEA) and Norway, was inaugurated at

Kjeller, near Oslo, on November 29. *Nora*, intended for the establishment of various physics data of importance for the development of power reactors, has been so constructed as to be very flexible and to permit a wide range of experiments to be carried out with it. One of its important features is the supporting device for the fuel elements which permits the fuel lattices to be changed readily. The reactor will operate at a power-level of only a few watts. The financing of *Nora* was made possible by a grant of 350,000 dollars from the U.S. Atomic Energy Commission under the Atoms for Peace programme in addition to the funds contributed by the Government of Norway.

The programme aims at obtaining precise and widely applicable reactor physics data on cores with mixed and variable lattices moderated by light water, by heavy water and by mixed heavy and light water. In addition to the critical assembly *Nora* itself, Norway is making available two reactor cores, one consisting of natural uranium and the other of slightly enriched uranium. A third core, provided under the supply agreement between IAEA and the United States, consists of fuel enriched to 3 per cent in the radio-isotope uranium-235. This core was originally used in development work for the nuclear merchant ship *Savannah* and contains approximately 42 kgm. of uranium-235. This is the first lease of special nuclear material through the Agency to one of its Member States. The Joint Scientific Programme Committee which is to conduct the *Nora* research project is headed by Dr. Raja Ramanna (India). The other committee members are Mr. Olav R. Kåsa and Dr. Henrik Ager-Hanssen, both from IFA, and Dr. Pierre Balligand, deputy director-general, and Dr. Carlo Salvetti, director of the Division of Research and Laboratories, both of IAEA.

Diamond Jubilee of Trans-Atlantic Radio

On December 12, 1901, Guglielmo Marconi became the first to send a wireless signal across the Atlantic. This remarkable achievement with such primitive equipment marked the birth of world-wide communication. During the spring of 1900, Marconi had succeeded in sending reliable signals from St. Catherine's in the Isle of Wight to the Lizard in Cornwall, a distance of 186 miles. This encouraged his belief that by using larger aerials and far more powerful transmitters he would be able to achieve trans-Atlantic distances. Scientists were highly sceptical; many said it was impossible because of the curvature of the Earth. Marconi was determined to make the attempt and a transmitting station nearly one hundred times more powerful than any previously constructed was built at Poldhu, near Mullion, in Cornwall. Enormous aerials were erected at Poldhu and at Cape Cod in Massachusetts, but both were wrecked in severe gales. Another, less ambitious in design, was put up at Poldhu, while Marconi and his two assistants sailed to Newfoundland where, from the top of Signal Hill, a receiving aerial was hoisted, at the third attempt, by means of a kite. At 12.30 p.m. (Newfoundland time) on December 12, 1901, Marconi and his assistant, G. S. Kemp, using one of the primitive receivers of the period with a telephone earpiece, heard a faint succession of S's in Morse code. Signals from Poldhu, 2,200 miles away, had crossed the Atlantic.

To commemorate this historic achievement, a Special Exhibition will be displayed at the Science Museum, South Kensington, during December 13–January 25.

Research in Shipbuilding

In a written answer in the House of Commons on November 21, the Parliamentary Secretary for Science, Mr. Denzil Freeth, stated that the shipbuilding and marine engineering industries in Britain have set up an executive committee, with the encouragement of the Department of Scientific and Industrial Research, to form a new association for ship research, and the proposals agreed within the industries were to be announced. Discussions with the Department on constitutional matters were well advanced, and those on details on the final financial arrangements are to follow.

U.S. Research Activities in the Antarctic

The U.S. National Science Foundation has announced a further allocation of approximately 1,500,000 dollars for research work in the Antarctic. The largest single grant among the twenty-seven grants which this sum comprises is for the Antarctic Meteorological Research Program of the U.S. Weather Bureau. The total grant for this programme is now nearly 400,000 dollars. A further sum, exceeding 100,000 dollars, has been allocated to a series of meteorological rocket firings. These firings will reach heights up to 200,000 ft. to investigate winds and temperatures. There has previously been no systematic measurements of these elements in high southern latitudes. The firings will be coordinated with similar soundings in other parts of the world, through the Meteorological Rocket Network, and the results will be correlated with world-wide stratospheric wind measurements. The U.S. Weather Bureau will also conduct an ice survey of antarctic waters with the assistance of U.S. Navy aircraft. This is intended to assist in the analysis of data to be received from projected polar satellites. A biologist from Ohio State University will carry out research while being based for sixteen months at the main U.S.S.R. base at Mirny. The main objective is to study invertebrate land animals and correlate the results with ecological studies which have been made at United States stations. This arrangement will continue the practice whereby the United States and U.S.S.R. exchange scientists at antarctic bases through agreements reached between the U.S. National Academy of Sciences and the Academy of Sciences of the U.S.S.R.

The Institute of Reprographic Technology

The Institute of Reprographic Technology was inaugurated at a reception held at St. Ermin's Hotel, Westminster, on November 23. The Institute will function as a professional body with entrance by examination, and its aims will be to increase the efficiency and technical ability of those practising the art of reprographic technology. This will be achieved by providing a forum for the exchange of ideas and a continuing programme for the dissemination of the scientific knowledge related to the profession. It is hoped that as the Institute grows regional organizations will be developed based on the industrial centres. Mr. Charles Hanrott has been elected chairman of the Institute. Further information can be obtained from the Honorary Press Officer, Institute of Reprographic Technology, 157 Victoria Street, London, S.W.1.

Impact of Science on Society

Impact of Science on Society, No. 3, 1961 (Vol. 11), includes several particularly interesting articles. An

editorial note, "Mankind and Outer Space", and an article by Prof. R. Courant, "General Problems confronting Computing Centres", both stress the opportunities and the importance of international co-operation in these fields. Dr. G. H. O. Burgess, of the Torry Research Station, reviews the contribution which fish technology can make to the food supply of the world and stresses the need for a much greater research effort and more attention to the dissemination of the results of research. Dr. G. Charlesworth, now head of the London Traffic Management Unit, Ministry of Transport, writing on "Science and the Road Traffic Problem", describes some of the contributions which research has made in this field, and the opportunities which the scientific study of roads and traffic presents both in the prevention of accidents and improving the efficiency of transport.

Education and Training in France

In May 1960 nine members of the West Regional Group of the British Association for Commercial and Industrial Education paid a visit to France to study the French system of education and training with special reference to the needs of industry. Their report contains a comprehensive description of the present system of education and training in France, as well as the changes in the system which will take place during the next few years as a result of legislation enacted in 1959 (Pp. v+51. London: British Association for Commercial and Industrial Education, 1961. 10*s*.). When reforms are completely integrated, all children will remain at school until sixteen years of age and selection for vocational training will commence well before the end of the normal school career. A large section of the report is devoted to the various types of vocational institutions which exist, the certificates and diplomas awarded and educational practice in the vocational schools. There is also a detailed description of education and training at the three main factories of the Renault Works.

Television and School Teaching

In a booklet on schools television, the National Union of Teachers suggests that television should not be considered as a substitute for the teacher but as an addition to teaching aids already available (*Schools Television: a Discussion Document*. Pp. 18. London: National Union of Teachers, 1961. 1*s*.); that if television is to be regarded as an integral part of the apparatus of education, it should be the responsibility of local education authorities to provide sets; and that particular attention should be directed to the need for the provision of more television sets in primary schools. The booklet quotes some of the findings from the Union's inquiry on schools television among 111 schools, distributed over the areas of 71 local education authorities, and compares them with similar findings in the B.B.C. report. The National Union of Teachers welcomes the setting up by the B.B.C. and commercial companies of special bodies with responsibility for the form and content of the schools programmes, and comments favourably on the "degree of independence of the parent body" which the Schools Broadcasting Department of the B.B.C. and its counterparts in commercial television enjoy. The booklet examines the constitution of these bodies, the scope of their work, the special role of the Union therein, and their relationships with the schools. The pamphlet states that "the principal limitations of the television programme compared with the film . . . are that it is not under the control of the teacher to the same extent either before, during or after showing, and that it has not the same resources of colour presentation". On the other hand, it points out that "television has a great impact on account of its immediacy". The booklet also touches on the general impact of television viewing on children and the special position of Wales with regard to television.

The British Broadcasting Corporation

In the annual report and accounts of the British Broadcasting Corporation for 1960–61 it is recommended that the higher-frequency bands, Bands IV and V, should be used for television with a change of line standard from 405 to 625 lines, as proposed by the Postmaster-General's Television Advisory Committee, and that two of the four channels available in the higher-frequency bands should be allotted to the B.B.C. One channel would be used for an entirely new programme service so that the B.B.C. could give viewers a genuine choice of balanced alternative programmes, while a second channel would carry the existing Band I service, duplicated on the 625-line standard. The B.B.C. recommends that the channels in Band III, not at present allocated to either B.B.C. or the Independent Television Authority, should be used to improve the coverage of existing services in areas not well served at present, and, in particular, to provide an improved and distinctive service for Wales. The B.B.C. is carrying on with its experimental work on colour television with the approval of the Post Office and with support from the radio industry. The experiments will include test transmissions in colour on 625 lines in Bands IV and V, thus extending the work already done by the B.B.C. in the experimental transmissions on 625 lines in black and white. On sound broadcasting, the report states that there is need for an earlier start to the day's programmes . . . a similar extension is needed at the other end of the day, after midnight, for late-night workers. Over and above the provision of three programme services, the B.B.C. sees the need and the opportunity now for a supplementary fourth service in the form of local sound broadcasting on very high frequencies.

Licentiateships in Printing and Furniture

For some time both the printing and furniture industries, in association with the City and Guilds of London Institute, have been developing advanced courses at a level beyond the full technological certificate to meet specific requirements for high-level qualifications for which, at present, there are no recognized awards. The Institute has announced recently a new award, the licentiateship of the City and Guilds of London Institute, intended to provide that kind of recognition in selected fields where such a need exists. The Institute has approved two such fields, printing and furniture, and the awards will be known as the Licentiateship in Printing of the City and Guilds of London Institute and the Licentiateship in Furniture of the City and Guilds of London Institute. Candidates for either award must have had at least five years approved industrial experience after completion of apprenticeship. In printing, a candidate will be required to have obtained one of the Institute's craft certificates, have obtained the full technological certificate and have passed the examination for advanced qualifications. In furniture, a candidate must have obtained a craft course

certificate, the full technological certificate and passes in two of the advanced courses.

The Dunfermline Museum

THE Dunfermline Museum was opened in June by Dr. Douglas Allan, lately director of the Royal Scottish Museum, Edinburgh. This new museum has been established by the Carnegie Dunfermline Trust and will serve as a local museum of natural history and folk material. The collections came mainly from the old museum at Pittencrieff House, which has now been closed. The conversion of a private house has provided two main galleries which have been equipped with modern 'shop window' cases. One of the galleries is given over to a display of the birds and animals of West Fife in their natural habitats, and the other shows material relating to Dunfermline from the stone age to the present day. A smaller room is devoted to linen, for many years the staple industry of Dunfermline.

South Australian Museum

THE *Records of the South Australian Museum*, **14**, No. 1 (1961), maintains the high standard of this publication and includes papers on the Aboriginal hammer-stones of South Australia, Acarina fauna, Central Australian mammals, the stratigraphy and vertebrate fauna of the Tirari Desert, South Australia, and a report on the archæological excavation of the Noola rock shelter.

Experimental Alteration of the Speech-rate

IT is known from the work of Dr. Wilder Penfield and his associates in Montreal that speech may be arrested or otherwise disturbed by electrical stimulation of certain areas of the cerebral cortex. In a recent paper from the Neurosurgical Service of the Foch Hospital (Suresnes-Paris), entitled "Arrest of Acceleration of Speech evoked by Thalamic Stimulation in the Course of Stereotaxic Procedures for Parkinsonism" (*Brain*, **84**, 363; 1961), Dr. G. Guiot and his collaborators have been able to show that striking changes in rate of speaking may be produced by thalamic stimulation in the course of stereotaxic operations for Parkinsonism. Although arrest of speech is stated to be the more common phenomenon, striking acceleration in the speech-rate was noted in certain cases and changes also occurred in the strength and modulation of the voice. The locus of stimulation is reported to be the ventrolateral nucleus of the thalamus. Although these observations call for repetition on the more adequate scale, they would appear to throw an interesting light on the part played by subcortical mechanisms in the delivery and control of speech.

Mineral Resources of Uganda

THE important part played by geological surveys in the economic advancement of under-developed territories is brought to mind by a recent publication on *The Mineral Resources of Uganda* (Bulletin No. 4: Compiled and edited by Dr. J. W. Barnes. Pp. v+89+2 plates. Entebbe: Geological Survey, 1961. Shs. 25). During 1960 the mineral commodities exported from Uganda reached a record value of £3·5 million, equal to almost a quarter of the aggregate output since mining first began in 1927; and in addition industrial minerals exceeding £1 million in value were produced for local use, and cobalt concentrates valued at £1 million were stockpiled. The principal products are blister copper and ores of tin, tungsten and beryllium; but within the next year there will be a large output of superphosphate (estimated at £325,000 per annum) for East African use and possibly of niobium ores for export. Since the Geological Survey began drilling for water in 1931 some 3,000 producing boreholes have been completed, yielding water supplies that have supplanted the disease-laden and ephemeral surface sources on which much of the population used to depend. In addition to reviewing resources now being exploited, the publication records various mineral potentialities of possible future interest, one of the more unusual of these being heavy water, which forms 8–12 per cent of the water in some volcanic lakes in the Ankole district. Although much of the country has still to be thoroughly prospected and, particularly in the north, to be mapped geologically, the report is a striking refutation of the view, once frequently voiced by Colonial administrators, that Geological Surveys are not revenue-producing departments.

Chagas' Disease

PRELIMINARY surveys on the incidence of Chagas' disease in the Matalaque region, Southern Peru, by Eleazar Cordova, have shown that housing conditions favour the development of *Triatoma infestans*, the only vector found in this zone. Domiciliary infection shows a high incidence (89·02 per cent), the rate of trypanosome infection in triatomides being 51·28 per cent (41·97 per cent in bedrooms), a fact that suggests the almost certain existence of human cases of Chagas' disease. Domestic animals are frequently kept and raised within houses; such is the case, for example, of guinea pigs (84 per cent), where *T. cruzi* was found in 4 out of 32 specimens examined. Felix Naquira, Eleazar Cordova, Cesar Naquira and Enrique Rubin de Celis (*Bulletin Chileno de Parasitologia*, **16**, No. 3; July–September 1961) have also carried out epidemiological surveys on Chagas' disease in the village of Matalaque (Tambo Valley, Southern Peru). The application of xenodiagnosis revealed an incidence of 34·67 per cent (43 out of 124 individuals surveyed) of *Trypanosoma cruzi* infection, whereas complement fixation procedures yielded 32·23 per cent positive cases (30 out of 93 individuals). Xenodiagnosis was also positive in four guinea pigs and one dog. The results obtained with the two methods were not entirely concurrent.

Fellowships in Engineering Study and Fundamental Research in Plastics

PRINCETON UNIVERSITY is offering a number of fellowships in engineering study and fundamental research in plastics leading to the M.Sc. and Ph.D. degrees, particularly suited to chemical, electrical and mechanical engineers, and to chemists and physicists. Instruction covers basic theory and properties, and principles of evaluation, production, fabrication, design and application of materials, as well as the chemistry of plastics. The programme includes lecture and laboratory classes, and contact with industrial plants representing various interests of the plastics industry. Fellowships with stipends valued at 1,500 dollars–2,100 dollars are available to applicants holding a first degree in engineering or physical science from a recognized institution. Further information can be obtained from Prof. Louis F. Rahm, Plastics Laboratory, Princeton University, Princeton, N.J.

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The Manchester College of Science and Technology

THE annual report of the Principal of the Manchester College of Science and Technology for 1960–61 gives an impressive picture of expansion, achieved and projected, which demonstrates how important a contribution the colleges of technology are making to Britain's scientific and technological strength. The number of students increased by 164 to 1,983, of whom 1,535 were studying for the B.Sc. (Tech.), 192 for the M.Sc. (Tech.) and 129 for the Ph.D.; 5,408 students were also enrolled for non-university courses, and of these only 333 were full time. The teaching staff now numbers 287. A list of research projects in progress during the year, arranged under Departments, appended to the report, gives an impressive picture of the research effort, and there is also a list of publications during the year by members of the staff. No information as to expenditure on research is included other than the statement that 9 per cent of the income of £1,580,523 was in support of research. Expenditure rose by £167,662 to £1,574,826. Students obtaining the B.Sc. (Tech.) with honours numbered 281, compared with 180 for the period 1959–60 and 96 for 1956–57; for B.Sc. (Tech.), ordinary, the corresponding figures are 178, 128 and 77; for M.Sc. (Tech.), 71, 59 and 33; for Ph.D., 25, 27 and 32; and for the postgraduate diploma, 22, 24 and 22.

University News:

Cambridge

CORPUS CHRISTI COLLEGE is planning a graduate extension of the College at Leckhampton House, Cambridge. It is intended to provide public rooms, and accommodation for a few Fellows, in the existing house, and to house research students and other Fellows in a new building close by. With this additional accommodation the College will be able to increase the number of its active Fellows from 22 to 37 or more, and its research students from 40 to at least 60. Prof. H. C. Longuet-Higgins, professor of theoretical chemistry in the University, has been appointed warden.

Edinburgh

DR. M. E. DAVIES has been appointed as lecturer in the Department of Veterinary Bacteriology, and B. F. Leek has been appointed as lecturer in the Department of Veterinary Physiology.

London

DR. B. W. LACEY, reader in bacteriology in Westminster Medical School, has been appointed to a chair of bacteriology at that School. Mr. D. G. MacRae, reader in sociology in the London School of Economics and Political Science, has been appointed to the chair of sociology tenable at that School. Dr. E. A. Power, lecturer in mathematics in University College, has been appointed to the readership in applied mathematics tenable at that College. Mr. J. L. Stollery, lecturer in the Imperial College of Science and Technology, has been appointed to the readership in aeronautics tenable at that College. The title of professor of molecular spectroscopy has been conferred on Dr. A. G. Gaydon, honorary lecturer in the Imperial College of Science and Technology.

Prof. J. T. Randall, Wheatstone professor of physics at King's College since 1946, has been transferred by the University of London to the chair of biophysics at the College. The lease of premises in Drury Lane, W.C.2, some 40,000 sq. ft. in extent, has been acquired to house the new Department of Biophysics. After conversion, the premises will be known as the Wellcome Biophysics Laboratories in recognition of the grant of £120,000 made by the Wellcome Trust in 1960 towards the purchase of the lease. Prof. Randall will assume the directorship of the Laboratories on their completion in 1963. The new premises will also house the Medical Research Council's Biophysics Research Unit, which has been an integral and important part of the biophysics activities of King's College since 1947.

Massachusetts

A HIGH-SPEED digital computer has been given to the Massachusetts Institute of Technology by the Digital Equipment Corporation of Maynard, Massachusetts. The computer is a "Programmed Data Processor", manufactured by the Digital Equipment Corporation, and will be used in teaching students the principles and operation of modern electronic data-processing equipment

Rhodesia and Nyasaland

DR. A. D. KEMP, senior lecturer in chemistry in the University College of Fort Hare, has been appointed lecturer in organic chemistry in the University College of Rhodesia and Nyasaland.

Announcements

SIR DOUGLAS LOGAN, principal of the University of London, has been appointed vice-chairman of the Association of Universities of the British Commonwealth in succession to Sir Eric Ashby. The chairman for the year 1961–62 is Mr. K. J. Maidment, vice-chancellor of the University of Auckland, New Zealand, and the honorary treasurer of the Association is Prof. R. Ogilvie Buchanan, of the London School of Economics.

DR. RANDAL M. ROBERTSON has been appointed as associate director (research) of the National Science Foundation, Washington. Dr. Robertson has been assistant director for mathematical, physical and engineering sciences at the Foundation since July 1958, a post which now passes to Dr. Geoffrey Keller.

MR. R. M. ARNOLD, until June of this year chief veterinary research officer in Tanganyika, has been appointed deputy head of the Wellcome Veterinary Research Station. Mr. Arnold became assistant veterinary investigation officer at Reading in 1942. Later he was appointed as veterinary investigation officer, Jamaica, and became deputy director of agriculture (veterinary and livestock) and superintendent of livestock services in that Colony.

THE 1962 International Conference on "Precision Electromagnetic Measurements" (formerly Conference on "Standards and Electronic Measurements") will be held during August 14–16 at the Boulder Laboratories of the National Bureau of Standards, Boulder, Colorado. Further information can be obtained from Dr. James F. Brockman, National Bureau of Standards, Boulder, Colorado.

THE International Society of Soil Science Joint Meeting will be held at Massey Agricultural College, New Zealand, during November 13–22, 1962, with pre-meeting tours during November 2–12 and post-meeting tours during November 23–December 1. The Joint Meeting is one of Commissions IV (Soil Fertility) and V (Pedology) of the International Society. Further information, together with an attractive brochure, can be obtained from the Secretary-General, P.O. Box 8001, Wellington, New Zealand.

THE TROPICAL PRODUCTS INSTITUTE

THE Tropical Products Institute of the Department of Scientific and Industrial Research held its first open days during October 19–20 when more than 500 people accepted invitations to be present. These included representatives of diplomatic missions; scientists from other Government institutions, universities and industry; representatives of commercial firms dealing in tropical products; and visitors from tropical countries. During the open days most of the laboratories were open to visitors and special displays were arranged to describe work in progress. This varied from the short-term advisory work to long-term research projects.

The Tropical Products Institute began as a part of the Scientific and Technical Department of the Imperial Institute and, therefore, has a history going back nearly seventy years. During most of the post-war years it was attached to the Colonial Office, but in 1959 was transferred to the Department of Scientific and Industrial Research, primarily in order that its services could continue to be available to Colonial territories after they had attained independence. In 1960 a total of nearly 1,000 inquiries was received from 62 Colonial, Commonwealth and foreign countries. These covered aspects of all the subjects on which specialists' advice is available at the Institute, including foods, fruits, essential oils, plant drugs, pesticides, oilseeds, fats, waxes, leather production, fibres, pulp and paper-making, board-making and solar energy. A comparatively new section at the Institute, the importance of which is growing considerably, is that dealing with inquiries on the economics of the production and marketing of tropical products; it also advises on suitable small industries for under-developed areas.

A research project with which the Institute has been concerned for many years is that of the pyrethrum industry in Kenya. Scientists at the Imperial Institute were instrumental in introducing this crop into Kenya in 1928 and it is now a very important export. One of the lines of work at present in progress is directed to improving the strains of the plant by breeding, and investigations are going on with Rothamsted Experimental Station to estimate the individual toxicities of the four esters responsible for the insecticidal activity of pyrethrum. These are separated by two-stage chromatography and sent to Rothamsted for testing. Another part of the Institute's work on pyrethrum is the investigation of the biosynthesis of pyrethrins in the plant. In particular, work has been directed at finding the site of synthesis and, for example, the effect of fertilization of the flower head. For this purpose acetic acid or mevalonic acid labelled with radioactive carbon is fed to the plant, the pyrethrins are isolated and, if radioactive, the site of labelling is determined. From this work it has been found that the synthesis occurs in the flower head and that fertilization of the flower does not influence the production of the insecticide.

One of the important projects in the Oils, Fats and Waxes Section has been an investigation into the factors which make Nigerian palm oil difficult to bleach. As a large part of the palm oil produced is used for margarine it is essential that it should be easily bleached to remove the reddish coloration which is normally present. Malayan palm oil has always been more easily bleached, and therefore has fetched a higher price, than palm oil from Nigeria. Work at the Institute has shown that the poor bleaching qualities of the Nigerian oil are due to the high initial carotene content and to oxidation caused by delays in processing which occur when primitive methods of collection and extraction are used. Oxidation by lipoxidase has been shown to take place when unsterilized and bruised fruit is left for long periods before extraction, and atmospheric oxidation during processing also takes place, usually due to iron contamination, where the iron acts as a catalyst. As in much of the Institute's work a fundamental chemical investigation has been followed by practical recommendations, and the small-scale producers of palm oil in Nigeria are now being encouraged to give more attention to the preparation and processing of the oil.

The accommodation at the Tropical Products Institute includes a pilot-plant laboratory which was on show to visitors during the open days. Here wax was being extracted from banana skins on a fairly large scale in a continuous process using about 7 l. of solvent. Heat is supplied by a steam-heated glass coil. The wax, which is removed by this method, constitutes 8–9 per cent by weight of the banana skin and may prove to be of use in the formulation of polishes. Investigations are also being carried out of the esters of a triterpenoid alcohol present in the wax. Also in the pilot plant laboratory are stills for the hundredweight-scale extraction of essential oils from bark, leaves and other plant materials. These provide very useful information for overseas growers who wish to produce these oils under larger-scale conditions.

In the Fibres Laboratory visitors were shown methods used for the physical testing of fibres, including a hybrid sisal which has been developed in Tanganyika. This has a much greater yield than the normal variety, and at the Institute the physical characteristics of the two types are being compared. Initial tests have shown that the hybrid gives a finer fibre and compares favourably in other respects with ordinary sisal. Other work in this Laboratory involves a study of the structure of fibres using methods similar to those used in electron microscopy. A cellulose acetate replica of the surface of a longitudinally cut fibre bundle is made, and this is metal-shadowed with chromium under high vacuum. The preparation is then examined using a light microscope and magnification of about 1,000.

The Pesticides Information Service of the Department of Technical Co-operation is also housed at the Tropical Products Institute and its work was shown to visitors during the open days. Much of the work in London is in providing information and abstracts, but there is also a laboratory where the control of bilharzia disease is being studied. This disease is caused by a parasitic worm and spread by various tropical snails. A colony of one of these, *Australorbis glabratus*, is maintained at the Institute so that promising chemicals can be tested as molluscicides.

Among the many other exhibits of work in progress, visitors were able to see types of building board made

of waste materials such as groundnut shells, work on the formulation of insecticides for the malaria control campaign of the World Health Organization, the study of the flavouring constituents of pineapple using gas chromatography, and the analysis of the amino-acid content of animal feeding stuffs.

The open days gave many people an opportunity to see something of the wide range of scientific work which the Tropical Products Institute is doing on the renewable resources of the less-developed countries of the world, for producers and users alike.

B. R. Smith

NEW INSTRUMENT ASSEMBLY WING OF THE CAMBRIDGE INSTRUMENT CO., LTD.

A NUMBER of scientists, industrialists, and well-known scientific and engineering journalists were among the 150 guests who attended a luncheon on October 23 to mark the opening of a new wing at the Cambridge Works of the Cambridge Instrument Co., Ltd. The guests were met by Dr. P. Dunsheath, chairman of the company's Board of Directors, and Mr. H. C. Pritchard, managing director, and the opening ceremony was performed by Sir Keith Joseph, M.P., Minister of State, Board of Trade.

After luncheon they toured the new wing, the Research Block which has been in use for two years, and Production Departments producing instruments for use in medicine and gas analysers for research and industry. In addition, a number of research projects and special displays of instruments made by the Company's two London factories were exhibited.

The new wing—which is 150 ft. long, 50 ft. wide and provides, on two floors, a working area of approximately 15,000 sq. ft.—is primarily intended to accommodate production departments for the final testing and assembling of instruments prior to dispatch. Immediately obvious features are the clean functional sweep of its two raised storeys, the ground-floor-level car-park and loading bay beneath, and the harmonious way it blends with the research laboratories, opened two years ago (see *Nature*, **184**, 1364; 1959), to which it has been planned as a logical architectural extension, and to which one end is joined by connecting doors. The other end of the wing is connected to other works buildings and the loading bay by a link block containing stairs and toilet facilities.

Structurally, the reinforced concrete frame of the raised building rests on a solid concrete slab supported by two rows of circular columns along its length, over which the slab is cantilevered out about 8 ft. front and back. The second floor and roof are mounted on pre-cast pre-stressed concrete beams spanning the full width of the building, so that the floor areas of both storeys are uninterrupted by intermediate columns. Windows are double glazed and the infill panels below faced with golden buff bricks. Air supplied throughout the building is conditioned and filtered by plant contained in two penthouses on the roof. Essential services, ventilating and heating inlets, gas, compressed air, water, electrical supplies, telephones, etc., are laid in horizontal ducts running the full length of the building below the windows and, to reduce fire risks, all work benches are wired with mineral-insulated metal-sheathed conductors.

Two-thirds of the first-floor area, adjacent to the entrance hall, is used as Production Offices and the remainder is occupied by the Microanalyser Department. This Department is responsible for assembling and testing 'Microscan' X-ray analysers and for the production of sealed and flow-type proportional counters. It is also concerned with the commissioning and servicing of 'Microscans' and operates an advisory service, including the analysis of samples provided by prospective customers. Complete installations are assembled in specially designed cubicles, each of which is supplied with its own special services. Conditioned air supplied to this Department undergoes an additional degree of filtration in view of the need for absolute cleanliness during final assembly of a 'Microscan'.

Fig. 1. Instrument assembly wing of the Cambridge Instrument Co., Ltd.

The whole of the second floor is devoted to final assembling, wiring and testing of a wide range of galvanometric indicators, recorders and controllers. Here again a dust-free atmosphere, so necessary for the assembly of intricate precision instruments and components, is provided. The functional interior design with its modern décor makes the new building a pleasant and comfortable place in which to work.

The new wing is the latest illustration of the considerable

development of the Company in recent years and has been made necessary by the increasing numbers of new and improved instruments developed by the Research Department. The building, which was designed by Edward D. Mills and Partners, architects, was completed in less than a year by the contractors, J. Jarvis and Sons, Ltd.

J. G. HAMMOND

LIFE AND ITS ENVIRONMENT IN ANCIENT SEAS

DURING the British Association meeting at Norwich, the Geology and Zoology Sections (C and D) held a joint session on "Life and its Environment in Ancient Seas".

Prof. C. M. Yonge, president of Section D, was in the chair and about 150 persons attended. The programme was designed as a general session covering a wide range of palæoecological topics, but unfortunately the opening address by Prof. Kaare Strøm (professor of limnology, University of Oslo), entitled, "In Quest of Ancient Seas", and the paper by Dr. R. H. Cummings (Department of Geology, University of Glasgow) on the "Palæoecology of Foraminifera in Reef Limestones" were both cancelled, and so the scope of the meeting was more restricted than originally planned.

The paper by Dr. K. A. Joysey (Department of Zoology, University of Cambridge) on "The Life of Cephalopods in Ancient Seas" was a discussion of existing views on the mode of life of fossil forms, considered in the light of new information about living cephalopods. Dr. Anna Bidder (Department of Zoology, Cambridge) showed a series of excellent colour slides of the living *Nautilus* and gave an account of her observations, largely based on freshly captured specimens living in the aquarium at Noumea, New Caledonia. The paper by Mr. R. W. Sheldon and Dr. F. M. Broadhurst (Department of Geology, University of Manchester) and Dr. J. G. Potts (United Steel Companies, Ltd.) on "Aspects of the Palæoecology of Marine Faunas in the Upper Carboniferous of the Southern Pennines", was a preliminary account of work still in progress seeking to elucidate the relationship between certain fossil faunas (mostly goniatites, lamellibranchs and brachiopods) and the petrology of the enclosing sediment. Hence, the session as a whole had an emphasis on molluscs, particularly cephalopods. Although unplanned, this bias came as a natural sequel to the molluscan theme of Prof. Yonge's presidential address.

Many 'palæoecological' publications (including some by the present author), are merely records of association and distribution, and offer little interpretation of the relationship between the fauna and the environment. The appreciation of a fossil biological system demands not only the reconstruction of the animals themselves but also of how they lived, what they did, their effects on other animals and on the environment. The Norwich session served well to illustrate this palæobiological approach to palæoecology.

In surveying the problems of buoyancy control, Dr. Joysey noted that the majority of living cephalopods are more dense than sea-water and support themselves by swimming, but *Sepia*, *Spirula* and *Nautilus* have a density near that of sea-water, owing to gas enclosed in rigid-walled chambers. Unlike the swim-bladder of a fish, the volume of the cephalopod float is not affected by changes in pressure. Hence, so long as some mechanism prevents the hydrostatic pressure from forcing liquid into the chambers through the porous siphuncular tube, changes in depth should not affect the buoyancy. Denton and Gilpin-Brown have recently shown that *Sepia* can adjust its buoyancy by pumping liquid in and out of the chambers, like a submarine altering ballast. This liquid has a lower salt concentration than the body fluids, and so osmotic pressure helps to counteract hydrostatic pressure. Dr. Joysey observed that as *Spirula* and possibly *Nautilus* are recorded from depths below that at which an osmotic mechanism would fail, some other mechanism must be sought if the gas is to be maintained in the chambers. An increase in the gas pressure would prevent liquid from flowing into the chambers and also prevent implosion of the shell, but any other mechanism whereby the siphuncular tube might become temporarily impermeable to liquid must be accompanied by a shell sufficiently strong to withstand the external pressure.

Dr. Bidder showed some photographs of *Nautilus* in a state of nearly neutral buoyancy swimming in mid-water or resting on the bottom, and others of it floating with part of the shell breaking the surface of the water. She had also observed under-buoyant animals hanging by their tentacles, and had found that individual specimens could adjust their buoyancy.

Dr. Bidder stated that the gas in the chambers was not under pressure in a specimen just hauled from a depth of nearly 200 metres. She had also extracted quantities of liquid from the gas chambers of *Nautilus* and using X-ray photographs she demonstrated that when a specimen had been turned upside down the shadows which were presumed to be bodies of liquid had indeed run to the opposite side of the chambers. She found no evidence of living tissue in the gas chambers of *Nautilus*.

In reviewing present-day interpretations of the posture of various fossil cephalopods, Dr. Joysey noted that the position of the gas chambers in orthoconic shells at first sight suggested that they hovered vertically in the water, or rested on the sea-floor like church steeples. Alternatively, if a load were present at the apical end, such as liquid in the apical chambers, they could have adopted a horizontal posture. It is generally accepted that, in some forms, this apical counterbalance was provided by solid deposits within the siphuncular tube. Other forms have deposits within the apical gas chambers, but there has been some controversy as to whether these were laid down by a cameral mantle or whether they are post-mortem mineral infillings. In view of the recent findings on living forms, Dr. Joysey suggested that serious consideration should be given to the possibility that these deposits might have been precipitated during life from solutions transported through the walls of the siphuncular tube.

By analogy with orthoconic nautiloids, Dr. Joysey pointed out that one of the functions of the belemnite

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guard was to act as a counterbalance. It lay at the posterior end of the main gas chambers while the bulk of the animal was at the anterior end, so giving a 'see-saw' type of stability. The morphological series from the belemnite to *Sepia* is well known. Functionally, this change involved sliding the body mass under the gas chambers, giving an 'air-ship' type of stability. In consequence, the counterbalance was progressively reduced and hence the volume of gas required to provide support could also be reduced.

Dr. Joysey referred to some palæozoic nautiloids (for example, *Ascoceras*) in which the juvenile was orthoconic, but by the development of highly oblique septa the adult achieved a horizontal posture, without resort to a counterbalance. Coiling of the shell produces a similar result as the body chamber comes to lie more or less horizontally below the float. Dr. Joysey briefly described Trueman's elegant investigation on the buoyancy and posture of ammonites. Trueman showed that an uncoiled form such as *Lytocrioceras* possessed greater stability than its tightly coiled counterpart. In functional terms such a shell has achieved the 'air-ship' type of stability, independently attained in such distantly related forms as *Ascoceras* and *Sepia*.

Dr. Bidder stated that *Nautilus* appears somewhat clumsy in side view but when seen from above, below or behind, its outline is remarkably streamlined. Its movement is slow in the encumbered environment of the aquarium, but more rapid in open water. Although it frequently swam near the bottom of the aquarium with its tentacles 'searching' the substratum, it had never been observed to crawl. Some of the photographs clearly showed the remarkable flexibility of the tip of the funnel, which can send the jet in almost any direction, thus giving considerable manœuvrability.

Dr. Bidder stated that the density of the body of *Nautilus* appears to be somewhat lower than Trueman's calculated value, and she suggested that it might prove necessary to modify his conclusion that the majority of ammonites were under-buoyant. Furthermore, his interpretations of the posture of ammonites are amenable to modification if the chambers contained unequally distributed fluid ballast.

Dr. Broadhurst outlined the work of Dr. J. G. Potts on the *Gastrioceras cancellatum* marine band near the top of the Millstone Grit Series, the work of Mr. R. W. Sheldon on another marine band in the Edale Shales towards the base of the Millstone Grit, and his own work in the Coal Measures.

In Dr. Pott's marine band some of the goniatites are preserved flat on their sides, whereas others are crushed in a vertical position, just as they settled on the sea-floor, leaving impressions of the venter on the bedding planes. It was suggested that a high proportion of vertically preserved goniatites probably indicated quiet conditions, and this was found to be at a maximum near the middle of the band, where the goniatites themselves were most abundant. The lamellibranchs had two periods of abundance, one above and the other below the goniatite maximum. The grain size and the amount of coarse detritus both had high values at the top and the bottom of the band, prior to the first and after the second lamellibranch peak. The marine band as a whole was described as a 'palæontological sandwich', the goniatites being the filling, the lamellibranchs the butter, and the coarse detritus the bread. It appeared that the absence of fauna in the sediments above and below the marine band could be attributed to turbulence and the greater rate of terrigenous sedimentation.

Mr. Sheldon's band contained fewer goniatites and lamellibranchs but abundant brachiopods. The amount of detrital material was low, most of the sediment being ankerite and silica. The brachiopods *Chonetes* and *Martinia*, the goniatite *Eumorphoceras* and the gasteropods in the fauna were abundant in the upper and lower parts of the band and sparse in the middle, analogous in pattern to the lamellibranch distribution present in Dr. Pott's marine band. In contrast the brachiopod *Productus* was abundant near the middle of the band and diminished upwards and downwards, this pattern corresponding with the abundance of ankerite.

Dr. Broadhurst was largely concerned with the conditions of sedimentation between marine bands. He showed photographs of a tree-cast 38 ft. high, standing vertically in non-marine sediments, its base rooted above a coal seam and its top in mudstone. He stressed that vertical preservation is not to be regarded as a freak occurrence, for he has records of many such trees at several horizons. Such preservation can only be interpreted as indicative of rapid sedimentation and it is significant in this respect that these vertical trees never cut through marine bands. Viewing their work as a whole Dr. Broadhurst suggested that a slow rate of deposition of terrigenous material appears to have been an important controlling factor during the deposition of the relatively thin marine bands, whereas more rapid sedimentation appears to have characterized the thicker intervening beds.

In the discussion which followed the papers Dr. Broadhurst and his colleagues indicated that the onset and the ending of marine band conditions were transitional rather than abrupt. Lateral changes could be detected at some horizons, for some *Lingula* beds pass laterally into true marine bands in other regions.

Dr. Joysey agreed that partially uncoiled ammonites like *Lytocrioceras* must have adopted different postures at various stages during growth, and commented that these might reflect changes in its feeding requirements. He noted that it was widely stated in the palæontological literature that *Nautilus* could adjust its buoyancy by moving in or out of its shell. Changes in shape of the body mass, however, would not affect its volume and so the density of the body would remain constant. Assuming that any space vacated within the body chamber would be filled with sea-water, then movements of the body would not affect its overall density. It appeared to him that this mechanism could not produce changes in density unless there was an unsealed gas bubble within the body chamber. Dr. Bidder stated that there was no gas bubble between the animal and the last septum, and that although small bubbles were sometimes present in the mantle cavity, these could be attributed to the aquarium aeration system.

The session as a whole provided useful liaison between geologist and zoologist, and each was able to gain a greater appreciation of some of the practical difficulties faced by the other in obtaining sufficient information to give an unequivocal answer to an apparently simple question. It also served to illustrate that one class of animals alone could provide abundant material for discussion, and that there remained plenty of scope for future joint sessions.

K. A. Joysey

THIRD AUSTRALIAN SPECTROSCOPY CONFERENCE

THE third Australian Spectroscopy Conference was held in the School of Chemistry at the University of Sydney during August 22–24 under the sponsorship of the Australian Academy of Science Spectroscopy Committee. A varied and interesting programme, consisting of 5 invited review papers and 51 research papers, was provided for the 227 participants. In addition, six exhibitors displayed some of the latest developments in spectroscopic equipment. The papers were arranged into sessions on vibrational, theoretical, molecular electronic and resonance spectroscopy with some parallel sessions concerned with instruments and analytical applications, including atomic absorption spectroscopy. After Prof. R. J. W. Le Fèvre had briefly welcomed the participants of the Conference to the School of Chemistry, Prof. N. S. Bayliss officially opened the proceedings on behalf of the Australian Academy of Science and welcomed to the conference the overseas guests, Prof. W. C. Price (King's College, London) and Mr. J. E. Allan (Rukuhia Soil Research Station, Hamilton, New Zealand).

The conference opened with a session on vibrational spectroscopy, and Prof. W. C. Price commenced with a stimulating review of recent developments in the far infra-red. After briefly referring to the experimental difficulties associated with investigations in the range 1–500 cm.$^{-1}$, he then proceeded to discuss the selection of sources, dispersing materials and detectors, and concluded by giving examples of the use of this region to obtain information on rotational energy-level in gases, lattice vibrations in crystalline and molecular crystals, and impurity energy-levels in semi-conductors.

Three research papers then followed dealing with infra-red studies on hydrogen bonding. Prof. A. N. Hambly (Department of Chemistry, School of General Studies, Australian National University) discussed the infra-red evidence for hydrogen-bonding in amino-sulphones, and concluded that stronger interaction occurs between the amino and sulphone groups on adjacent carbon atoms than occurs in the corresponding nitro-amines. This was attributed to the difference in the geometry of the interacting groups. Mr. P. E. Rogasch (Weapons Research Establishment, Salisbury) produced evidence to show that proton transfer can occur in systems that are strongly hydrogen-bonded. Mr. I. H. Reece and Prof. R. L. Werner (Department of Physical Chemistry, University of New South Wales) dealt with the intermolecular interaction between carbonyl and hydroxyl groups in solution, and showed how this interaction can be used to deduce *pKa* values for the hydroxyl compounds from the infra-red spectrum.

The session continued with papers on analysis of the vibrational spectrum on mono-methyl-arsine (Dr. V. Cranmer, Imperial Chemical Industries, Australia and New Zealand, Ltd., Central Research Laboratories, Ascot Vale), of dichlorofluoromethanesulphenyl chloride (Dr. N. S. Ham, Division of Chemical Physics, Commonwealth Scientific and Industrial Research Organization) and tri-methyl borate (Prof. R. L. Werner, Department of Physical Chemistry, University of New South Wales). Dr. E. Spinner (Department of Medical Chemistry, Australian National University, Canberra) discussed the vibrational spectra and structures of the hydrochlorides of the aminopyridines. The Raman and infra-red spectra suggest that whereas the positive charge resides on the ring nitrogen in 3-aminopyridine it is located on the amino-nitrogen in 2- and 4-aminopyridine. Mr. R. H. Laby and Dr. D. J. Greenland (Waite Agricultural Research Institute, University of Adelaide) were concerned with the implications of the infra-red spectra of molecules of amino-acids, simple peptides, alcohols and glycols adsorbed by montmorillonite. Methods for increasing the discrimination between similar infra-red spectra were described by Dr. J. Tregellas-Williams (Defence Standards Laboratories, Maribyrnong). Progress being made in the development of improved microwave-powered Raman sources was summarized by Dr. N. S. Ham and Dr. A. Walsh (Division of Chemical Physics, Commonwealth Scientific and Industrial Research Organization). The session on vibrational spectroscopy was concluded with a paper by Dr. P. Marmet (Division of Chemical Physics, Commonwealth Scientific and Industrial Research Organization) dealing with the problem of producing monoenergetic electrons in a mass-spectrometer and their use in obtaining details of the vibrational structure in the energy states of simple molecular ions.

Dr. N. S. Ham (Division of Chemical Physics, Commonwealth Scientific and Industrial Research Organization), in the session on theoretical and molecular electronic spectroscopy, reviewed some theoretical and experimental aspects of the Jahn–Teller theorem. He pointed out how the experimental manifestations of the theorem depended on the magnitude of the distorting forces. Examples showing orbital and/or spin degenerate ground and excited states were discussed. Mr. E. F. McCoy and Dr. I. G. Ross (Department of Physical Chemistry, University of Sydney) considered the change of shape in electronic transitions of polycyclic aromatic hydrocarbons. They showed that encouraging agreement is obtained with experiment if calculations of the overall width and intensity distribution in the corresponding emission or absorption spectrum are based on the sum of the squares of the bond-length changes that accompany the electronic transition. Mr. J. C. Mackie and Dr. L. E. Lyons, from the same department, outlined theoretical methods which enable the accurate calculation of the ionization potential of a molecular crystal from a knowledge of the ionization potential of the free molecule, its polarizability and the crystal structure. Prof. W. C. Price discussed the ionization potentials of some simple radicals and molecules in terms of their electronic structure. It was shown that the π electron orbitals of the halogen substituted CH_3, CH_2 and CH radicals account for the magnitudes of the observed ionization potential and stability of parent molecules and ions. The effects of progressive substitution on the ionization potential of ethylene were also explained.

Dr. E. G. McRae spoke on the theoretical relationship between fluorescence and vibrational structure and Davydov splitting in molecular crystals. Application of his theoretical treatment to the available information on the crystal fluorescence spectrum of

anthracene leads to a prediction of a Davydov splitting of the order of 500 cm.$^{-1}$. It is interesting to compare this with a later paper in the conference in which Mr. A. R. Lacey and Dr. L. E. Lyons (Department of Physical Chemistry, University of Sydney) described experimental investigations of the Davydov splitting in the spectrum of anthracene crystals which yielded an estimate of ~ 330 cm.$^{-1}$ for this splitting; the much lower values (0–60 cm.$^{-1}$) usually reported are attributed to the combined effect of incomplete polarization of the incident radiation and the thickness of the crystals used by other investigators.

Mr. M. Batley and Dr. L. E. Lyons (Department of Physical Chemistry, University of Sydney) discussed refinements in the quantitative prediction of the crystal spectrum of phthalocyanine. In a paper by Dr. E. A. Magnusson and Dr. H. Shull (Australian Missionary College, Cooranbong) it was suggested that the barrier to internal rotation about a single bond may be explained both with regard to height and sense, in terms of interactions between the electrons in the terminal bonds and also to the possibility that the distribution of the electrons in the single bond itself is not cylindrically symmetrical. Mr. G. L. White (Defence Standards Laboratories, Maribyrnong) presented a theoretical paper on polarization in Raman spectra. Dr. A. D. Buckingham (Chemistry Department, Harvard) spoke on spectroscopy with polarized radiation. He discussed the existing methods of determining the direction of the transition dipole moment with respect to molecular axes and outlined a new approach involving the measurement of the Kerr constant through absorption bands.

A review by Dr. I. G. Ross (Department of Physical Chemistry, University of Sydney) on optical masers preceded the session on molecular electronic spectroscopy. After explaining the basic principles of optical masers, Dr. Ross considered some of the general and research applications possible for the monochromatic high-intensity beams that can be produced. Dr. T. M. Spotswood (Division of Coal Research, Commonwealth Scientific and Industrial Research Organization) presented evidence for the formation of charge transfer complexes during the bromination of forms of carbon known to contain relatively high proportions of aromatic structures. The existence of charge-transfer complexes between brominated polycyclic hydrocarbons and halogens in solution was also established, and the spectra of these were compared with those for the corresponding complexes for the corresponding unsubstituted hydrocarbons. In a contribution from Dr. A. V. Bree (University College, London) the fluorescence and absorption spectra in the near ultra-violet of crystals of 9,10-dichloroanthracene, measured at room temperature, were discussed and shown to confirm that the corresponding transition in crystalline anthracene is short-axis polarized. Dr. Bree showed that photo-produced impurities accounted for fluorescence and photo conductivity observations. In another paper from the same department, Dr. T. M. Dunn presented the results of a rotational analysis of the 2700 Å. system of *para*-difluorobenzene which confirm that a $B_{2u} \leftarrow A_g$ transition (polarized along short axis, that is, in plane of benzene ring and perpendicular to *F–F* direction) is responsible.

Three papers from the Weapons Research Establishment, Salisbury, were concerned with investigations of the character of the emission associated with various electronic transition processes in flames and in the upper atmosphere. Dr. C. L. Cook and Mr. J. A. McFarlane described the spectral characteristics of the emission from an ammonia/bromine pentafluoride diffusion flame. Mr. L. J. Drummond and Mr. P. E. Rogasch did the same for the active nitrogen–chloroform and active nitrogen–methylene chloride flames. Dr. J. C. Burns outlined some of the difficulties associated with attempts to measure temperatures from the AlO-radical emission which accompanies the re-entry of the main body of a rocket research vehicle into Earth's atmosphere. Vibrational equilibrium appears to exist and temperatures in the range 1,600°–2,000° K. were obtained.

During the session on analytical applications on emission spectroscopy Mr. L. S. Dale and Mr. R. N. Whittem (Australian Atomic Energy Commission Research Establishment) described the spectrographic techniques in use in health surveys of beryllium contamination. In a second paper, Mr. Whittem discussed the spectrochemical analysis of toxic and α-active materials. Mr. A. B. Timms (Australian Mineral Development Laboratories, Adelaide) spoke on the spectrographic determination of hafnium/zirconium ratios. Mr. J. M. Nobbs (Defence Standards Laboratories, Finsbury) presented evidence to show that while the input of electrical energy to an arc gap between metal electrodes can be controlled to within very fine limits, the output of radiation from the arc column varies randomly from pulse to pulse by factors as large as 200, and is caused primarily by fluctuations in the evaporation of electrode material. The theory of the photographic characteristic and its use in plate calibration formed the subject of a paper by Mr. C. Candler (South Australian Institute of Technology). The analysis of lubricating oils for the additive elements barium, phosphorus, calcium and zinc with a direct-reading spectrograph was outlined by Dr. R. A. Cummins (Defence Standards Laboratories, Maribyrnong). Mr. I. Collison and Mr. T. Scott (Broken Hill Pty., Newcastle, N.S.W.) spoke on their experiences with the calibration of an A.R.L. 'Quantovac' for the analysis of sulphur and phosphorus in carbon and low alloy steels.

In a session on instruments and techniques the following papers were presented. Mr. J. J. McNeill (Division of Chemical Physics, Commonwealth Scientific and Industrial Research Organization) discussed factors influencing the design of an inexpensive general-purpose monochromator for use above 2000 Å. The construction and performance of an Ebert scanning spectrometer was discussed by Dr. S. C. Baker (Physics Department, Newcastle University College, N.S.W.). A photoelectric device to prevent slow image shifts during long exposures in a high-resolution échelle spectrograph was described by Dr. G. R. Hercus (Division of Chemical Physics, Commonwealth Scientific and Industrial Research Organization). Outstanding problems of instrumentation in the development of the full potentials of X-ray fluorescence spectroscopy as an analytical tool were briefly considered by Mr. J. E. Maloney (Philips Electrical Industries, Sydney). Mr. J. F. Horwood and Mr. D. A. Forss (Dairy Research Section, Commonwealth Scientific and Industrial Research Organization) described methods used for handling micro-quantities of samples for absorption spectrometry. Mr. F. B. Wunderlich (John Lysaght Pty., Ltd., Newcastle, N.S.W.) indicated how low-resolution infra-red spectra could be used for the identification of constituents in paints and plastics. Mr. A. T. Martin (C.S.R. Chemicals, Rhodes) spoke on the reflectance of opaque materials as a function of pig-

ment concentration. Finally, Dr. N. Kelso King (Division of Physical Chemistry, Commonwealth Scientific and Industrial Research Organization) discussed the significance of isosbestic points in studying reaction mechanisms.

The session on atomic absorption spectroscopy, which was held on the final morning, proved to be one of the most successful sessions of the conference. It was very gratifying to witness the obvious intense interest in this subject, which was pioneered in Australia and New Zealand. Mr. J. E. Allan (Rukuhia Soil Research Station, New Zealand) opened this session by reviewing recent developments in the technique. The progress made since the second Australian Spectroscopy Conference in 1959 was amply illustrated by the increase in published research papers from 5 to 50 at present. Dr. B. G. Davey (School of Agriculture, University of Sydney) described the application of atomic absorption spectroscopy to the analysis of agricultural materials. It is now possible to determine rapidly many elements in soil and plant material; up to 170 samples an hour can be analysed for one element with a coefficient of variation less than $\pm$ 5 per cent using very simple equipment. Mr. D. J. David (Division of Plant Industry, Commonwealth Scientific and Industrial Research Organization) discussed the determination of molybdenum by atomic absorption. Molybdenum is believed to be present in a flame as the oxide under normal conditions, but in an acetylene–air flame operated under reducing conditions the molybdenum trioxide dissociates to atomic molybdenum which can be detected by absorption. Mr. David suggested that compound formation under flame conditions may account for many of the interferences that can occur in analysis by atomic absorption. The research in atomic absorption being carried out at present at the Rukuhia Soil Research Station was surveyed by Mr. J. E. Allan. Extension of the range of elements accessible to the technique by the use of nitrous oxide–acetylene flames is being investigated. The determination of cobalt and nickel in agricultural materials has been studied in detail, and procedures established for the analysis of fertilizers. Dr. J. B. Willis (Division of Chemical Physics, Commonwealth Scientific and Industrial Research Organization) showed how atomic absorption was being used to determine traces of heavy metals such as lead, mercury, bismuth, nickel, cadmium and zinc in biological materials. The sample preparation procedures are much simpler than those involved in most existing methods; cadmium and zinc can be determined directly in urine and preliminary experiments suggest that zinc may be determined in blood serum and whole blood by spraying the diluted material directly into the flame. Cathodic sputtering appears to be an attractive method for atomizing those elements (for example, aluminium, strontium and titanium) which form refractory compounds under flame conditions, and Drs. B. M. Gatehouse, J. V. Sullivan and A. Walsh (Division of Chemical Physics, Commonwealth Scientific and Industrial Research Organization) discussed the factors affecting the cathodic sputtering of various metals. In some instances it has been possible to establish sputtering conditions which permit analytical determinations, but the method is still unsatisfactory for aluminium.

The final session of the conference was devoted to resonance spectroscopy. Dr. G. S. Bogle (Division of Physics, Commonwealth Scientific and Industrial Research Organization) reviewed the development of the theory of electron paramagnetic resonance. Mr. A. J. Harle, Dr. L. E. Lyons and Mr. B. K. Selinger (Department of Physical Chemistry, University of Sydney) summarized the results of a preliminary survey of the electron-spin resonance spectra of some charge-transfer complexes between chloranil and aluminium oxide, hexamethyl benzene and tetracyanoethylene, dibenzacridine and tetracyanoethylene or chloranil. Dr. G. S. Bogle (Division of Physics, Commonwealth Scientific and Industrial Research Organization), Drs. W. F. Forbes and W. E. Savige (Division of Protein Chemistry, Commonwealth Scientific and Industrial Research Organization) reported some investigations of the electron paramagnetic resonance properties of cystine and related compounds after irradiation with mercury 2537 Å. radiation and a sun-lamp (peak ~ 3100 Å.). Similar spectra are obtained for all the compounds examined, indicating that the same trapped free radical $RS\cdot$ ($R = CH_2{\cdot}CH{\cdot}(NH)_2{\cdot}COOH$) is formed in each case on irradiation. Mr. T. J. Seed (Department of Physics, University of Canterbury) discussed the paramagnetic spectra of manganous ions in undiluted crystals of the solid-solution ammonium chloride–manganous chloride–water.

In the final paper of the conference Dr. C. K. Coogan (Division of Chemical Physics) and Prof. H. S. Gutowsky (Chemistry Department, University of Illinois) reported on nuclear magnetic resonance in sodium hydrosulphide. Observations on the hydrogen-1 and sodium-23 resonances in polycrystalline samples of sodium hydrosulphide tend to confirm Pauling's hypothesis that the hydrosulphide ion is tumbling very rapidly in the crystal lattice and thus presents an outward spherical form to the surrounding ions. This occurs above 80° C., and it was also observed that SH^- ions can diffuse through the lattice.

R. A. Durie

COLLOIDAL SYSTEMS

An informal discussion on colloids, under the auspices of the Faraday Society, was held in the Royal College of Science and Technology, Glasgow, on June 27. The meeting was held to mark the centenary of the appearance of Thomas Graham's paper, "Liquid Diffusion applied to Analysis", in which a clear definition of colloids and crystalloids was first given. An attempt was made to cover as many colloidal topics as possible; in the event, this did not prove feasible and the subjects tended to be somewhat biased towards association colloids. About seventy people attended the meeting, including several from Holland.

The chairman, for the morning session, was Prof. P. L. Pauson, who welcomed the visitors to the Royal College and indicated the reasons for holding the discussion at this time and place. (Graham held his first chair at the College.) The first speaker was Dr. A. J. Hyde (Glasgow), who gave a brief biography of Graham with rather greater emphasis on the private

and non-scientific side of his life than on his scientific achievements, which are reasonably well known.

Sir Eric Rideal (London) then gave an address on "Colloid Science: a Century of Progress". Sir Eric reviewed the development of colloid chemistry since 1861, commencing with Graham's work on peptization, gels and sols. He then discussed work on coagulation, flocculation and peptization of sols, drawing on the work of Von Weimarn, Hardy and Perrin for examples. A review of optical investigations on colloidal solutions was then given, reference being made to the work of Faraday and Tyndall, and finally to the ultramicroscope of Zsigmondy and Siedentopf. Gels and adsorption were dealt with rather more briefly, mention being made of the work of Freundlich and the swelling experiments of van Bemmelen.

The final speaker in the morning session was Dr. A. S. C. Lawrence (Sheffield), who gave a talk describing the work of his group at Sheffield on soap–water–amphipile systems. Graham did not carry out any investigations on soap solutions, but they come within his classification of colloids. Dr. Lawrence commenced with a general description of the colloidal and other phenomena encountered, and of the phase diagrams of the systems. He continued with a more detailed account of the liquid crystalline forms and myelinic figures which are obtained on adding soap solutions to crystals of a polar additive, or solutions of additive to a soap crystal. These were interpreted in terms of adsorption of soap and additive at the solid-liquid interface. In conclusion, mention was made of the effect of organic and inorganic salts on the systems.

For the afternoon session, the chairman was Sir Eric Rideal and the first speaker, Dr. A. P. Prosser (London). Dr. Prosser gave a talk on the forces which, in conjunction with electrostatic forces, stabilize all colloidal systems. Beginning with a general account of van der Waals forces and London dispersion forces he then went on to deal with their origin and the differences which exist when bodies are very close together (< 250 Å. apart, the unretarded case) or relatively far apart (> 2000 Å., the retarded case). He then gave a brief outline of the theory of the forces and the problem of evaluating them from the theory in the retarded and unretarded cases. The experimental determinations of attractive forces by the groups led by Bradley, Derjaguin, Overbeek and Kitchener were then discussed, along with some recent experiments on flocculation in colloidal systems.

The second paper in the afternoon, entitled "Polyelectrolytes", was by Prof. J. A. V. Butler and Dr. D. J. R. Laurence (London) and was read by Dr. Laurence. The subject-matter is directly connected with Graham's work on caramel and gelatin. Dr. Laurence mentioned the general properties of polyelectrolyte solutions such as high viscosity and gel formation. He then went on to discuss more specialized properties such as titration curves, conductivity curves and transport properties which give rise to the picture of a polyelectrolyte molecule in solution as a charged random coil with small gegenions, some of which are 'free' and some 'trapped' inside the coil. The last point is particularly well illustrated by measurements of transport number. The spatial configuration of the coils was then discussed in terms of the information obtainable from sedimentation, viscosity and light-scattering measurements. Finally, deoxyribonucleic acid was treated in rather more detail, followed by a mention of proteins, including ribonuclease.

The final speaker, Prof. J. T. G. Overbeek (Utrecht), spoke on the drainage of soap films. He began with a few general remarks on the structure of soap films, their thickness and colours. Then followed a description of the stretching of films, with the formation of new film, and the drainage of films. Finally, the nature of the black film was discussed, with reference to the discontinuity in thickness at the edge of the black area, the forces giving rise to the black film and the ultimate breaking stability of the film when electrostatic and van der Waals forces are opposed to one another. Prof. Overbeek then presented a colour ciné film of the drainage of soap films illustrative of all the points he had mentioned, and containing, in addition, several beautiful illustrations of moving colour effects obtainable in soap films.

A. J. Hyde

THE LIBRARY ASSOCIATION

ANNUAL CONFERENCE

THE papers presented at the annual conference of the Library Association at Hastings during September 19–22 were of general rather than specific interest to the scientist. Mr. Leslie Wilson and Viscount Caldecote made some reference to the National Reference Library of Science and Invention and the National Lending Library for Science and Technology and to the proposals for the re-organization of the Library Association, particularly as they seek to establish the Association as the professional association of librarians, and their papers are of direct consequence to scientists and technologists. Sir Charles Snow's lively presidential address, with its plea for a wide view of the functions of the public library in the Welfare State, should also commend itself to them. Sir Charles believes that the public libraries must in future assume some of the functions of the great private lending libraries and that, in particular, they have a real responsibility to contemporary literature which some of them have yet to recognize. Mr. K. J. Lea, county librarian of Essex, on the other hand, while not discounting this amenity aspect, to which the smaller library authorities are virtually limited, urged that more attention should be given to the educational and specialized services, if necessary by the development of a two-tier system based on co-operation between boroughs and counties, the possibility of which is already being demonstrated by the county libraries of Britain.

Mr. Leslie Wilson's paper, on "Libraries and Information Services: the Challenge of the Future", was equally concerned with co-operation, alike between such bodies as the Library Association and the Association of Special Libraries and Information Bureaux and between these and institutions concerned with the use of information. He stressed first

the desirability of library and information work being recognized generally as a single distinctive professional activity and then the relevance of such an activity to the fragmentation of knowledge. Effective special library work in any one field depends, he said, on co-operation between librarian and information officer and the specialist working in that field, and he suggested we have still far to go before the idea was generally accepted that accumulated knowledge, with the librarian as its vehicle, is as essential to industrial prosperity as capital investment, production and sales. Viscount Caldecote indeed went some way in this direction when, in his paper earlier that day, he suggested that the research worker can reasonably expect library staff to be responsible for two-thirds of his library searching. Mr. Wilson thought, moreover, that we must expect a much greater participation of the Government in library affairs, and for all these reasons it would be wise for librarians and their organizations to clarify their ideas as to the form of partnership in which they could function most effectively.

Mr. Wilson cited the plans for the new National Reference Library of Science and Invention as a glaring example of the confusion and frustration likely to result from failure to achieve such co-operation and consultation between user and librarian at the earliest stage; but he also viewed with concern the proliferation of professional bodies concerned with such a relatively small field as library and information work. Formal federation is probably impractical at present, but he suggested that some form of consultative committee or a series of consultative committees, each dealing with a special interest such as education or research, might help to achieve the closely knit community that is needed. The Aslib Groups, he suggested, seem to be a reasonably successful means of dealing with the problem, but of the seventeen organizations listed in the handbook of special librarianship and information work as active in this field in the United Kingdom, at least the Library Association, Aslib, the Circle of State Librarians, the Standing Conference of National and University Libraries, the United Kingdom branch of the International Association of Music Libraries, the Association of British Theological and Philosophical Libraries and the Institute of Information Scientists should come closer together.

Viscount Caldecote's paper expressed an industrialist's view and criticism of library and information services. He thought that the main industrial library problem centre around the small firm. He suggested first that the larger firms might make their own extensive library facilities available to these smaller firms, and that by doing so the trained library staff would enable the smaller firm to tap also the resources of the national libraries. Viscount Caldecote repeatedly stressed the importance of quality of trained library staff, and he also suggested that member firms could do more to strengthen the library resources and services of the Research Associations. He was impressed by the value of the area co-operative schemes formed in Sheffield, Newcastle upon Tyne, Liverpool and elsewhere, and in this connexion he thought the liaison thus established with educational bodies is of vital importance for the pooling of resources and knowledge. Finally, in discussing briefly the functions and staffing of industrial libraries, themselves, Viscount Caldecote suggested that the Association might form a committee to study the automation of routine library services.

Other papers presented at the Conference, by Dr. C. Bibby and by Mr. A. E. Sanderson, dealt with the functions of training-college libraries; Mr. B. Williams firmly challenged the exercise of censorship in reading by a local authority or other body. In his Library Association Annual Lecture, "The Fourth *R*", Dr. Lincoln Ralph reviewed the limitations of an education confined to reading, writing and arithmetic in coping with the modern world; he urged that it is necessary not simply to instruct but also to inspire. Education should impart a sense of values, of the purpose of life, and lead to a re-birth.

THE AUSTRALIAN ATOMIC ENERGY COMMISSION

THE eighth annual report of the Australian Atomic Energy Commission*, which covers the financial year ended June 30, 1960, records a year of substantial progress in the high-temperature gas-cooled reactor project, in the production and application of isotopes, and in the installation and commissioning of new equipment.

The report consists of nine sections dealing respectively with an outline of the activities of the Commission; the search for uranium; uranium mining; raw materials, including thorium and beryllium; research activities; isotopes; nuclear power; international agreements and co-operation with the International Atomic Energy Agency; and general activities. Details of research contracts, postgraduate research studentships and undergraduate scholarships, together with the financial accounts and the names of the staff of the Commission, are given in appendixes to the report.

Although the world's annual uranium production potential at present exceeds the demand, steadily increasing requirements for nuclear power stations are expected to develop from the 1970's onwards, and the Commission, through the Bureau of Mineral Resources, has continued its surveys for new deposits of uranium. Except for one area stated to show anomalous radioactivity, no discoveries of radioactive materials were reported to the Commission during the year, but a number of further monetary reward instalments were paid for earlier discoveries.

For the first time, complete production figures are given in the report for operations at Rum Jungle since the start-up in 1954. A record tonnage of ore was treated during 1959–60, and all the uranium oxide produced was delivered to the Combined Development Agency under the terms of the Rum Jungle Agreement, which expires on January 6, 1963. No mining was carried out during the year, and the treatment plant continued to operate on stockpiled ore.

Full-power routine operation of the reactor *Hifar* began in February 1960 after a prolonged period of testing and calibration. The reactor has given little trouble, and major irradiation work has been performed for the Commission, and under contracts

* Australian Atomic Energy Commission. Eighth Annual Report for the year ended 30th June, 1960. Pp. 63. (Coogee, N.S.W.: Australian Atomic Energy Commission, 1960).

with the United Kingdom Atomic Energy Authority and the General Electric Company. The materials irradiated included prototype fuels, beryllium and beryllium oxide samples, and cobalt for the production of cobalt-60.

New buildings in the Research Establishment were officially opened on May 27, by the Minister for National Development, the Hon. W. H. Spooner. They consisted of the canteen, adjoining the building provided by the Commonwealth Government as the headquarters of the Australian Institute of Nuclear Science and Engineering, and a building devoted to isotopes and technical physics. A 10-kW. graphite moderated reactor, named *Moata*, ordered from the United States of America, is to be installed in the new reactor physics building to be finished this year. *Moata*, which will have a maximum thermal neutron flux of 10^{11} neutrons cm.$^{-2}$ sec.$^{-1}$, is designed for flexibility of core and reflector arrangement, and will be used to measure thermal and resonance neutron cross-sections, to study slowing-down properties of moderators such as beryllium, and to investigate long-term changes in fuels and moderators. Studies of the feasibility of a reactor system require intensive investigation into nuclear materials, and the various aspects of the research into the fabrication of graphite, beryllium, beryllium oxide, and sample fuel elements; the effects of irradiation on selected materials; and the compatibility of each material with the others, are described in some detail in the annual report. For the study of beryllium and for work on solid-state physics in co-operation with the Commonwealth Scientific and Industrial Research Organization and Australian universities, two neutron collimators are in operation on *Hifar* for the production of neutron beams, a long wave-length neutron spectrometer has been installed, and two other spectrometers are being constructed.

During the period under review, Australian industry showed increasing interest in the adoption of radioisotope techniques for the solution of its problems, but the field for potential industrial applications is still largely unexplored. A second detailed survey of the extent of the applications of radioisotopes in Australian industry is being prepared, and information already available shows that a substantial increase since the first Commission survey in June, 1958, has occurred. Two changes were made in the Advisory Service: a direct consulting service to industry was initiated on a limited scale, and early in 1960 the Isotopes Section commenced making direct approaches to various firms. Seventy technical discussions were held with inquirers at the Research Establishment and members of the Advisory Service made ninety visits to factories and laboratories. In consultation with the Isotopes Section, the Commission's Engineering Services Design Group undertook the design of a cobalt-60 γ-irradiation unit for the study of plant mutations, and the long-term research programme includes the technical uses of radiation for insect pest control, sterilization of medical supplies and equipment, and food preservation by radiation. At the end of May 1960 the Commission sponsored a conference on the "Technological Use of Radiation". The three-day conference was attended by 165 delegates, and sixteen papers were presented and discussed (the proceedings are now available in book form from the Melbourne University Press, Parkville, Melbourne, N.2, or from the Commission).

The number of staff employed at the Establishment increased by 189 to 673 during the year. A few members of the Commission's scientific staff were stationed in the United Kingdom, United States of America and Canada, and an atomic energy attaché was appointed to the staff of the Australian Ambassador in Washington for closer working contact. The Commission provided £60,000 for the building housing the Australian Institute of Nuclear Science and Engineering which was opened in May, and gives £50,000 annually for Institute research and training. Although no undergraduate scholarships have been awarded since 1957, ten students are still undergoing training in geology, geophysics, metallurgy and chemical engineering. The students graduate this year. Forty-three research contracts have been awarded to eight universities and two hospitals.

MENTAL HEALTH

THE tenth report of the World Health Organization Expert Committee on Mental Health is devoted to a review of present mental health facilities and resources and to the establishment of priorities for a mental health programme on a world-wide scale*. Essentially it is based on a summary of work carried out in the field of mental health by the World Health Organization during 1949–59, and on the responses to an inquiry circulated to specialists in many countries in an attempt to ascertain what is being done and what needs to be done in that field.

The report first examines trends in mental-health work during the past decade, covering such subjects as mental health aspects of public health, psychiatry of childhood, treatment methods and facilities, organization of psychiatric services, education and training, and research. Under each heading attention is directed to the contribution made by investigations sponsored by the World Health Organization, particularly those reflected in the reports of previous Expert Committees and Study Groups.

An examination of the aims of mental-health work and of the ways of achieving them is followed by a discussion of psychiatric and mental-health services, their organization, planning and priorities. Emphasis is placed on the importance of training programmes, since "even the most efficient organization and the best physical facilities will not prevent a badly staffed service from soon becoming unsatisfactory". The training of each category of mental-health worker is dealt with in turn. In addition, an idea is given of the kind of education in mental health required for personnel from the other health professions, as well as for community leaders and the general public, so that all may help to promote the mental health of the community.

The report touches briefly on team work and describes the attitudes required in a team leader and qualities desirable in team members. Considerable space is given to research in the field of mental health

* World Health Organization. Technical Report Series. No. 223: *Programme Development in the Mental Health Field*. Tenth Report of the Expert Committee on Mental Health. Pp. 56. (Geneva: World Health Organization; London: H.M.S.O., 1961.) 2 Swiss francs; 3*s.* 6*d.*; 0.60 dollars.

and it is pointed out that, although investigations undertaken in various countries over recent years show encouraging results, the complexity of the problem makes progress slow. The areas of research considered of foremost importance in mental-health work are enumerated and discussed in detail. Among the many subjects recommended for investigation are studies on brain function, epidemiology, health and social studies of communities undergoing rapid change, the hospital *milieu*, ecology of mental illness, problems of ageing, the effect of nutrition on mental health, and genetics.

The report concludes with a consideration of the vital part that the World Health Organization can play in future planning for mental health, by fostering international co-operation and exchange of information, and by facilitating and stimulating research.

APPLICATION OF PALÆOMAGNETISM TO THRUST MECHANICS

By D. K. NORRIS and R. F. BLACK

Geological Survey of Canada, Ottawa

THE surface trace of the Laramide Lewis thrust fault in the south-eastern Cordillera of Canada exhibits a change in direction of approximately 35° from a north-west trend east of the Clark and Lewis Ranges to a north trend east of the Flathead Range (Fig. 1). The nature and genesis of this change of direction has been the subject of much controversy in recent years, and explanations have included differential erosion of the thrust plate and differential rotation of the plate. The purpose of this article is to show that palæomagnetic directions in conjunction with surface geological data suggest that the Lewis thrust plate moved as a coherent tectonic unit and that the gross geometry of the plate may have been established at the time thrusting was initiated.

For convenience in reference, the Lewis thrust plate will be divided geographically into two parts, termed respectively the Flathead Range segment and the Clark and Lewis Ranges segment.

These two parts are structurally and stratigraphically continuous with one another at their common boundary in the vicinity of North Kootenay Pass. Precambrian rocks of the Purcell system have been mapped without interruption between them, and no major transverse faults are there to suggest that the two segments of the Lewis thrust plate operated as independent tectonic units[1]. The continuity as well as the simplicity of the structure between the two segments would suggest rather that the plate moved as a single structural unit.

The change in trend of the surface trace of the Lewis thrust therefore must be due either to differential erosion of the plate, such that the Flathead Range segment has been eroded to some considerable degree farther west than the Clark and Lewis Ranges segment, or to the possibility that the latter segment was always structurally farther east than the former. The fact, however, that the fold and thrust fault elements in the Clark Range do not end abruptly at the margin of the plate where the surface trace of the Lewis thrust swings west but rather swing sub-parallel to it suggests that the re-entrant at North Kootenay Pass is not due to differential erosion of the Lewis thrust plate. The possibility of differential rotation, therefore, is especially impor-

Fig. 1. Location map

tant in the interpretation of the mechanics of emplacement of the plate.

If differential rotation has occurred it should be possible to determine the approximate amount of relative motion by a comparison of the directions of the remanent magnetic vectors obtained from Purcell samples collected in the two segments. A necessary condition, however, is that the time of magnetization of the rocks making up the plate be pre-Laramide (older than Early Tertiary) in order that the results obtained can be applied to this problem.

Fifty-nine samples of unweathered, highly quartzose, fine-grained, red sandstone were collected from the Precambrian Purcell system at intervals normal to the strike at three locations in the Lewis thrust plate (Fig. 1). They were from member *A* of the Kintla formation in the Flathead Range where the strata strike north, and, from members *A* and *C* of the same formation, at two localities in the Clark Range where the strata strike north-west.

The remanent magnetization in each of two cubes from each sample of Kintla formation was determined with a sensitive astatic magnetometer in the laboratories of the Dominion Observatory of Canada. From the measurements of two cubes the mean attitude of the remanent magnetic moment was computed for each sample.

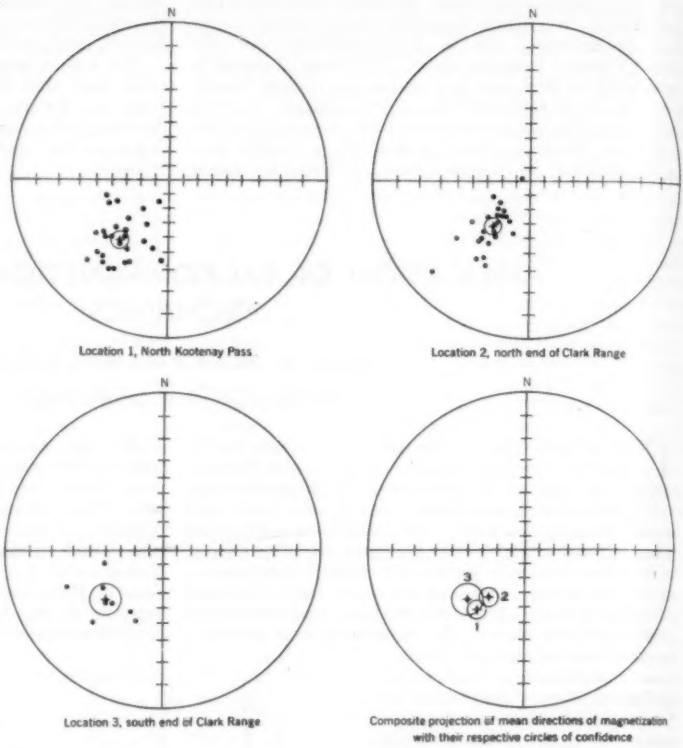

Fig. 2. Stereographic projections of the directions of magnetization for the three locations and composite projection of their respective mean directions and circles of confidence. ●, vectors pointing downwards; +, mean direction of magnetization

All cubes were subjected to decreasing-amplitude-alternating-field demagnetization and the maximum amplitude of the field was progressively increased after each set of measurements until no significant change (less than 5°) occurred in the magnetization direction in the cubes. This approximately stable state of magnetization was reached at 156 œrsteds.

Results from this demagnetization treatment indicate that the Kintla formation, where sampled, is magnetically stable, as the directions of magnetization for the majority of cubes were not significantly altered. After demagnetization the directions in the remainder became consistent with those on which demagnetization had little or no effect.

The mean palæomagnetic results for the three localities sampled are indicated in Table 1 and plotted in Fig. 2. Solid circles plotted on the Wulff stereographic projections in Fig. 2 represent the directions of the magnetic vectors with their positive pole pointing downwards. The mean direction for each group of samples is represented by a solid circle centred by a cross. The statistical parameters computed for the three groups of measurements are N, θ, and K as defined by Fisher[3], where N is the number of samples, θ is the radius of the circle of confidence calculated at the 95 per cent level of confidence (represented in Fig. 2 as open circles on the lower hemisphere of the stereographic projection), and K is a measure of the dispersion. All measurements are with respect to bedding, which was assumed to be horizontal at the time of deposition.

The fact that the calculated pole positions included in Table 1 agree well with those pole positions determined from Purcell and other Late Precambrian North American rocks[3,4] would suggest that the remanent magnetization has been preserved since Precambrian times. The results may therefore be applied with reasonable assurance to test for the possibility of differential rotation in the Lewis thrust plate in the early tertiary.

The directions of the magnetic vectors of samples from member *A* were in all cases found to point upwards and have been adjusted (sign of the inclination changed from negative to positive and 180° added

Table 1. Mean Palæomagnetic Results for Individual Collecting Sites

Collecting site	Location 1 North Kootenay Pass	Location 2 North end of Clark Range	Location 3 South end of Clark Range
Formation and member	Kintla *A*	Kintla *A* and *C*	Kintla *A*
Polarity*	−	− and +	−
Number of samples	27	24	8
Mean declination	218·5°	218·0°	230·0°
Mean inclination	+39·5°	+49·0°	+38·0°
θ	5·5°	6·5°	9·5°
K	23	24	40
Ancient pole position Lat.	10·5° S.	3·0° S.	6·5° S.
Ancient pole position Long.	150·5° W.	146·5° W.	159·0° W.

* For comparative purposes all vectors have been adjusted to point downward.

to the declination) to point downward for comparison of results from members *A* and *C*.

It is evident from the composite projection in Fig. 2 that the circle of confidence for any one group of observations is intersected by those of the other two groups. This would suggest that within the limits of error of collection and measurement, which is between 5° and 10°, little if any relative rotation of the Kintla formation has occurred between any of the three collection sites. The differences in the mean declination angles are negligible between Flathead Range and the north end of the Clark Range and has a maximum of 12° between the latter and the south end of the Clark Range. It should be recalled that if the change in trend of the surface trace of the Lewis thrust were due entirely to differential rotation a disparity in the declination of the order of 35° should be indicated.

In summary, the continuity of surface structural and stratigraphic units throughout the length of the Lewis thrust plate in the area under investigation would suggest that the plate moved as a single, coherent, tectonic unit with no major transcurrent or transverse normal faulting. Palæomagnetic data, moreover, support this contention as there was, within the limits of error, no measurable differential rotation of the plate between the Flathead and the Clark and Lewis Ranges. It appears, therefore, that the Clark and Lewis Ranges were always structurally farther east than the Flathead Range. The present configuration of the eastern limit of the Lewis thrust plate is apparently genetically related to the geometry of the thrust surface established during the initial stages of movement rather than to differential erosion along the eastern margin of the plate.

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LOSS OF FLUORESCENT PARTICLES IN ATMOSPHERIC DIFFUSION EXPERIMENTS BY COMPARISON WITH RADIOXENON TRACER

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ZINC cadmium sulphide, disseminated in a finely ground particulate form, mainly in the size-range 1–5μ, has been widely used as a tracer in work on diffusion and atmospheric pollution. This fluorescent tracer technique was described independently by Perkins *et al.*[1] and Braham *et al.*[2] in 1952. The various problems in which it has been used can be conveniently divided into two groups. The first includes investigations of the spread of airborne substances released into the atmosphere. Here, only relative measurements of the concentrations of the particles are important in a particular trial, and provided any comparison of these is made after equivalent times of travel, then any time-decay of the tracer in a physical or chemical sense is unlikely to introduce error. The second is of particular importance in air-pollution investigations and includes all problems where the tracer is used to estimate the absolute concentration at any position resulting from a release of known size. Here, the decay characteristics of the tracer must be well defined.

Earlier workers[1,3] considered that zinc cadmium sulphide was stable in the presence of normally occurring concentrations of atmospheric pollutants for at least the first few hours after release. However, some work by one of the present authors (N. T.), to be reported elsewhere, in which vertical profiles of the particles were investigated some tens of kilometres down wind of releases on a long cross-wind line, showed an apparent decrease in the number of airborne particles with increasing distances of travel. A more reliable estimate of the loss was required, and it was decided to compare the fluorescent tracer directly with an inert gaseous tracer. Radioactive xenon-133 (half-life 5·27 days) was selected because of ease of sampling and detection.

The xenon and zinc cadmium sulphide tracers were released simultaneously at a uniform rate and sampled simultaneously at two distances down-wind. The total amount (number of particles or activity) of either tracer collected, divided by the sampling rate, is a measure of the dosage (time-integral of volumetric concentration) of that tracer at the point in question. If D_1, D_2 are the dosages of xenon at the near and far sampling distances respectively (units, μμc. min./m.³) and d_1, d_2 the corresponding dosages of particles (units, particle min./m.³) then L, the percentage loss of particles, is given by:

$$L = \left(1 - \frac{D_1}{D_2}\Big/\frac{d_1}{d_2}\right) \times 100$$

This result is independent of the position of either sampling station with respect to the plume axis.

On each of three days, approximately 10 kgm. of zinc cadmium sulphide (Derby Luminescents 1318/10) and 3 curies of xenon-133 were emitted from a 20-metre incinerator stack at the Atomic Energy Research Establishment, Harwell, over a period of about 2 hours beginning near noon. Calculation showed that the health hazard to a person situated at the point of maximum ground-level concentration was negligible. During the three trials reported here, the wind directions varied between south-west and west-north-west with mean speeds of between 4 and 7 m./sec. Vigorous convection extended to heights of up to 1,700 m. and there was about one-half cloud cover present.

Sampling of both tracers took place at two stations placed one about 16 km. and the other about 60 km.

Table 1

	June 23, 1961		July 7, 1961		July 25, 1961	
Amount of tracer Fluorescent particles (gm.) Xenon (curies)	$1{\cdot}442 \times 10^4$ 3·31		$1{\cdot}362 \times 10^4$ 3·65		$0{\cdot}983 \times 10^4$ 2·00	
Sampler distances (km.) Mean period of travel of tracers (hr.)	14·5 1·0	60·0 3·2	17·7 1·0	57·0 3·1	16·1 0·7	63·5 2·5
Dosages Fluorescent particles (particle min./m.^{3})	38,000	3,700	55,100	12,100	(*a*) 29,700 (*b*) 36,050	(*a*) 4,500 (*b*) 5,850
Xenon ($\mu\mu$c. min./m.^{3})	1,010	188	1,600	739	601	163
Dosage ratios Florescent particles (d_1/d_2)	10·3		4·5		(*a*) (*b*)	6·6 6·2
Xenon (D_1/D_2)	5·4		2·2			3·7
Loss of particles (per cent)	48		51		(*a*) (*b*)	44 40

(*a*), Assessment under 80 × microscope; (*b*), higher intensity ultra-violet illumination and 100 × microscope used in assessment.

from the source. A sampler was also operated about 1 km. up-wind of the source to measure the background activity of atmospheric xenon. Satisfactory positioning of the sampling stations in the plume of material was obtained through frequent pilot balloon measurements of the wind at Harwell, and by direct observation of the bearing of the plume axis a few kilometres from the source, using a mobile fluorescent particle sampler in conjunction with inspection equipment set up nearby.

The fluorescent particles were collected by drum impactors, through which air was drawn at a rate of about 20 litre/min. The drums were rotated through a small angle at 5-min. intervals to prevent a large accumulation of dust on the traces, which might have obscured a significant number of the impacted particles. In the subsequent assessment, the drums were illuminated with ultra-violet light and the particles counted through an 80 × microscope. The samples from the third trial were also assessed under ultra-violet light of higher intensity, with counting under a 100 × microscope.

The xenon-133 was collected by passing air from a petrol-driven compressor at a flow-rate of 100 litre/min. through a charcoal U-trap immersed in a dry-ice alcohol mixture at −78° C. The trap contained 400 gm. of activated charcoal and was preceded by a soda-lime column to remove carbon dioxide, a magnesium perchlorate drying column and a pre-cooling coil. The trap was followed by a warming coil and a rotameter.

After sampling was completed, the charcoal trap was returned to the laboratory where a measured volume, approximately 10 ml., of inactive carrier xenon was added. The trap was then heated slowly to about 200° C. and the xenon, together with some oxygen, nitrogen and other gases, was flushed with purified hydrogen into a smaller charcoal chromatographic column immersed in liquid nitrogen. The xenon was separated from other gases by a gas chromatographic technique using hydrogen as the eluting gas. A measured volume of the xenon, together with diethyl ether vapour as a quench, was transferred to a 50-ml. Geiger counter. This was operated in a low-background assembly with an anti-coincidence ring. Counter backgrounds were about 1·5 c.p.m. and the minimum sample count-rate approximately 10 c.p.m. The chemical purity of the separated xenon was established by mass-spectrometric analyses. Radon was the principal radioactive impurity expected and its absence was demonstrated by operating the counter in the proportional region to enable any radon α-pulses to be distinguished from the xenon-133 β-pulses, and also by following the decay of each sample for at least one half-life. Count-rates were corrected for radioactive decay to a standard time and an allowance was made for the xenon lost in processing and transfer to the counter. The correction due to xenon background activity did not exceed 20 per cent of the sample activity. The collection efficiency of the field sampling equipment, determined in separate experiments, was better than 85 per cent for periods up to 4 hours, and a small correction was made, appropriate to the duration of sampling after arrival of the xenon-133 at the sampling station. A full description of the xenon sampling technique will be published elsewhere.

The results are summarized in Table 1.

The mean loss of particles in travelling between the two sampling stations is seen to be approximately 50 per cent. A several-fold increase in the intensity of ultra-violet light used in the assessment of the fluorescent particle samples resulted in an increase of about 25 per cent in the number of particles counted in the last trial. However, the dosage ratios from the two assessments were almost identical and the corresponding values for the loss of particles differed by less than 4 per cent. The explanation for the loss is unknown at present, but it is relevant that separate studies of the size spectrum of the particles after different distances of travel showed an apparent decrease in the proportion of small particles with increasing distances of travel. Measurements[4] on particles collected in these last experiments showed that the fluorescent brightness of particles less than 2·5μ diameter decreased with increasing time of travel. To account for the loss by deposition, a velocity of deposition of 15 cm./sec. would be required, which is about 60 times larger than the value determined in wind tunnel experiments (Chamberlain, A. C., personal communication). In any event, this process would lead to a preferential loss of the larger particles.

Our thanks are due to D. V. Booker (Atomic Energy Research Establishment) for advice and assistance in counting the xenon samples, also to G. F. Collins and R. A. Titt (Chemical Defence Experimental Establishment) for assistance with certain aspects of the fluorescent tracer technique.

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STRUCTURE OF THE 5,6-DIMETHYLBENZIMIDAZOLYLCOBAMIDE COENZYME

By Dr. P. GALEN LENHERT* and DOROTHY CROWFOOT HODGKIN, F.R.S.
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RECENTLY, H. A. Barker *et al.* reported the isolation of a crystalline 5,6-dimethylbenzimidazolylcobamide (DBC) coenzyme[1]. This is one of a series of coenzymes which were first recognized as catalysts in the conversion of glutamate to β-methylaspartate in certain bacteria. The coenzyme was shown to be active in systems requiring vitamin B_{12}, but it differed from cyanocobalamin in having an adenine nucleoside in place of the cyanide group. We have now carried out an X-ray analysis of the coenzyme structure, having for our use crystalline preparations kindly given to us by Dr. H. A. Barker, Dr. Karl Folkers and Prof. A. W. Johnson.

Part of Dr. Barker's preparation was recrystallized by slowly diffusing acetone into an aqueous coenzyme solution. Two freshly grown crystals prepared in thin-walled glass capillary tubes in dim light and kept in contact with their mother liquor were used to obtain the X-ray diffraction data for the structure analysis. Copper $K\alpha$ radiation was used throughout. The intensity measurements were made visually from multi-film equi-inclination Weissenberg photographs taken about the *b* and *c* axes. The Lorentz-polarization correction and a spot shape correction were applied. The scale factor for each layer was calculated from reflexions common to other layers and the absolute scale was obtained by Wilson's method. 3,145 independent reflexions extending out to a spacing limit of 0·9 Å. were observed. These included about 85 per cent of the reflexions with a spacing greater than 1·2 Å.

The unit cell dimensions characteristic of the wet crystal lattice, as measured from precession photographs, are: $a = 27{\cdot}9_3$, $b = 21{\cdot}7_2$ and $c = 15{\cdot}3_4$ Å. The space group is $P2_12_12_1$ and the measured density of the fully solvated crystals, $1{\cdot}35_5 \pm 0{\cdot}01$ gm./cm.^{3}. The molecular weight, coenzyme plus solvent, calculated from this information is 1,899. This is larger, as would be expected, than the weight measured by Mattern on air-dried crystals[2]; the difference corresponds to the weight of about 8 molecules of water of crystallization.

The structure analysis began with the calculation of a sharpened three-dimensional Patterson synthesis which clearly showed the Co—Co vectors. The first electron-density synthesis was computed using phases calculated from the cobalt positions and the observed *F* values weighted according to a method suggested by Sim[3]. The resulting electron-density map contained peaks representing most of the atoms in the structure, and in addition, spurious peaks, many of which were larger than those corresponding to atoms. The position of the cobalt atom ($z = 0{\cdot}02$) produced a pseudo-mirror plane which distorted the electron-density pattern representing the corrin nucleus and increased the number of spurious peaks. In spite of this, reasonably accurate positions were worked out for nearly all the atoms in the corrin nucleus, the 5,6-dimethylbenzimidazole, the ribose and the phosphate group.

A second electron-density synthesis, again using Sim's weighting scheme, was computed with phases calculated from the 54 atoms with known positions. This synthesis, containing less false symmetry and fewer spurious peaks, enabled us to place nearly all the remaining atoms of the coenzyme molecule. The electron-density in the region above the corrin nucleus, on the side occupied by cyanide in cyanocobalamin, is of particular interest (Fig. 1). The adenine moiety can be clearly seen among the spurious peaks. It is evident as a planar set of peaks with the 5- and 6-membered rings and the amino-group correctly related. Further, the five nitrogen atoms, with one exception, correspond to the peaks of greater electron-density. The sugar part of the nucleoside is not so easily seen, but on careful study it is no less convincing. This arrangement of peaks represents a *d* ribose unit linked directly to the cobalt atom at C 5′. No peak can be found which would correspond to the hydroxyl oxygen of C 5′. Most of the spurious peaks are in chemically inadmissible positions and have disappeared from subsequent electron-density syntheses.

Any residual doubt about the correctness of the above interpretation was completely dispelled by the results of the next structure factor calculation and the appearance of the corresponding difference synthesis. All the atoms in the coenzyme molecule (except six in the vitamin B_{12} part) and three water molecules were included in the third structure factor calculation. The agreement factor, *R*, was thereby reduced from 0·44 to 0·28.

Agreement between the observed and calculated structure factors has been further improved by adding the remaining six atoms of the vitamin B_{12} part, by including additional solvent molecules and by making adjustments in the positional and thermal parameters

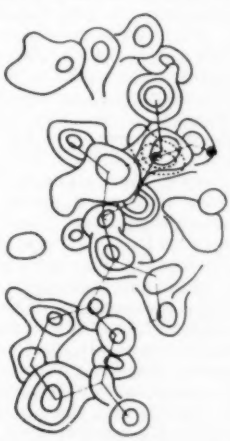

Fig. 1. Peaks in the second electron density synthesis in the region of the adenine and sugar groups. The contours are at levels corresponding approximately to 1·0, 1·5 and 2·0 $e/Å.^3$. The distribution is shown viewed along *a* and the contours are drawn only near the level of the peak maxima

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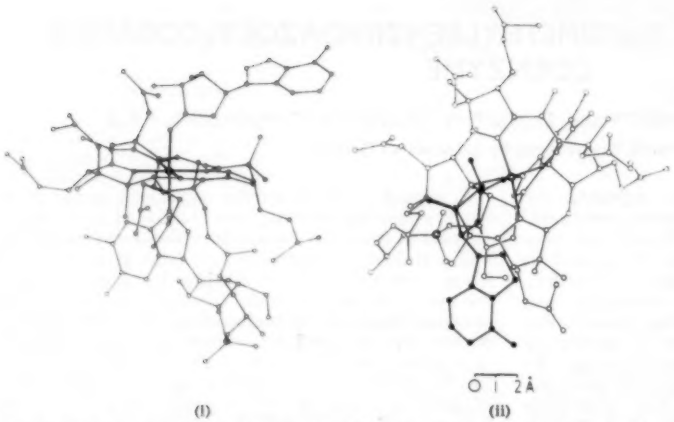
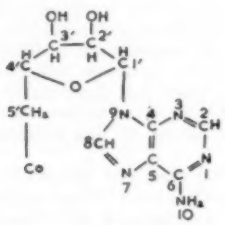


(I) (ii)

Fig. 2. The atomic positions in one molecule of the 5,6-dimethylbenzimidazolylcobamide coenzyme as found in the wet crystals and viewed along (i) the *b* axis, (ii) the *a* axis. In (ii) the atoms of the nucleoside unit are marked by filled circles

of the other atoms. All the atoms of the coenzyme molecule (excluding hydrogen) and contributions from 11 water molecules have been included in the latest structure factor calculation. The agreement factor is 0·173.

The structural formula of the nucleoside as it occurs in the intact coenzyme should almost certainly be written as in I, which represents it as an adenosine derivative.



(I)

The *d* ribose unit and the adenine are linked at C 1′ (β) and N 9 in accordance with the observations of Ladd *et al.*[4] The unsaturated sugar, CH_2 = CH.CHOH.CHOH.CHO, which these authors obtained by the action of cyanide or mild acid hydrolysis, must be formed on degradation of the coenzyme following the rupture of the Co—C 5′ bond. The observed tetrahedral character of the atomic centres C 2′, C 3′ and C 4′ makes it unlikely that a double bond involving any of these atoms is initially present in the molecule; and very recently, adenosine derivatives in which the pentose ring is intact and the 5′ CH_2OH is replaced by COOH and CHO have been isolated from among the photolysis products of the coenzyme by Johnson and Shaw and by Barker *et al.*[5].

Fig. 2 shows the arrangement of the atoms in the coenzyme molecule as found in this crystal structure. All the atoms of the vitamin B_{12} molecule appear to be present except the cyanide group which has been replaced in the coenzyme by the adenosyl residue. The cobalamin nucleus and the nucleoside are connected by the C 5′—Co bond of 2·05 ± 0·05 Å.; the Co—C 5′—C 4′ angle is rather large, 130°, which suggests that this bond may have partial ionic character. No other direct covalent or hydrogen bonds connect the corrin nucleus and the nucleoside. The closest non-bonded contact is 3·1 Å.

While several of the amide side-chains have adopted conformations different from those found in B_{12}, there is no evidence of any change in the corrin nucleus itself. Within the accuracy of the present partly refined co-ordinates, the corrin nucleus appears to be identical in the vitamin and coenzyme molecules. In the circumstances we could most easily formulate the cobalt atom as trivalent in the coenzyme molecule and write the structure in a similar way to that of vitamin B_{12}. The coenzyme molecule would then have a molecular formula of $C_{72}H_{100}O_{17}N_{18}PCo$ and a molecular weight of 1,580. The uncertainties here are in the number of hydrogen atoms, the valency of cobalt and the exact character of the bonding in the corrin nucleus; they may possibly be resolved by further refinement of the crystal structure, particularly if we can observe the hydrogen atom positions. Other evidence, notably on the magnetic character of the crystals, is very conflicting at present[6,7] and it seems likely that easy transformations occur within the molecule which involve the valency of cobalt and the corrin nucleus. These, combined with the unusual character of the carbon–cobalt bond, are presumably responsible for the catalytic activity of the coenzyme.

The crystal structure of the DBC coenzyme is, in detail, quite different from that found in the vitamin B_{12} crystal structures. Although the orientation of the corrin nucleus with respect to the crystal axes is similar, the relationship between the four molecules which make up the translational unit of the crystal has altered. In the new arrangement, very interesting intermolecular contacts arise which suggest methods of approach by different reagents in the neighbourhood of the Co—C 5′ bond. For example, the adenosyl 3′ OH is hydrogen bonded to the phosphate group of one neighbouring molecule while the NH_2 group of the adenine unit is in contact with the phosphate group of another. The geometrical relations strongly suggest easy pathways both for the formation of the coenzyme from adenosine triphosphate and for its possible intervention in nucleic acid synthesis.

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VARIANTS IN THE STRUCTURAL POLYSACCHARIDES OF ALGAL CELL WALLS

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ALTHOUGH the wall polysaccharides of the algae have been extensively investigated there are nevertheless many puzzling features which remain to be resolved. We have for this reason been making detailed examination of the walls of a wide variety of these plants, and it is the purpose of this article to present these newer results in the general context of the older, pending more extensive publication elsewhere.

It has long been recognized that the walls of plant cells consist of two phases, one a dispersed crystalline phase and the other a matrix which is commonly non-crystalline, both phases containing largely polysaccharides and/or polysaccharide derivatives. Since 1948 the dispersed phase has been identified in the electron microscope as consisting of long thin threads—the microfibrils—and it has become the custom, except in most fungi, to associate these with cellulose. In higher plants, the microfibrillar component usually occupies a large proportion of the wall volume so that the wall normally gives a characteristic X-ray diagram of cellulose I (native cellulose). In the algae, however, the relative volume of the crystalline wall material is often so small that the scatter from the amorphous incrusting substances may mask the X-ray diagram completely, and microfibrils may no longer be visible when chemically untreated material is examined in the electron microscope.

In 1952, Nicolai and Preston[1], using in the main the technique of X-ray diffraction analysis of untreated algae, divided the Chlorophyceae into three groups so far as wall structure is concerned. Group 1 consisted of the Valoniaceae and the Cladophoraceae (with the exception of the *Spongomorpha* group of the Cladophorae), algae containing highly crystalline cellulose I in abundance in the form of relatively straight and comparatively thick microfibrils. The bulk of the plants were placed in Group 2, characterized by X-ray diagrams resembling those of cellulose II (mercerized cellulose) and the rest, giving unidentifiable X-ray diagrams, made up Group 3. It was afterwards reported from this laboratory that the marine Phaeophyceae[2] and, with one exception, the Rhodophyceae[3] resemble in this regard the green algae of Group 2. The classification was regarded throughout as provisional because the interpretation of the X-ray diagrams of untreated algae in Groups 2 and 3 was scarcely ever unambiguous. This was due both to uncertainties inherent in the possible masking of reflexions by scatter from amorphous substances and to the presence in some diagrams of reflexions referring clearly to two different crystalline substances.

The major difficulties in the way of an identification of the crystalline component of algal cell walls lie partly in the need for careful removal of incrusting substances and partly in the invariable presence in marine algae of a deposit of micro-crystalline material on the plant surfaces[4]. This deposit, which is often present in large amounts, especially in filamentous algae, we have identified as a mixture of clay minerals. As it cannot be removed, even by the most severe washing, the X-ray diagram of untreated algae is often that of the clay minerals alone. It is undoubtedly because the strongest three reflexions of the mixture of minerals present lie very close to the strongest reflexions of cellulose II, both in disposition and in spacing, that cellulose II has been suspected to be the skeletal substance of Group 2 algae. Chemical treatment can reduce this contamination to an amount usually so small as to allow the diagram of the crystalline wall polysaccharides to be recognized. In particular, the treatment used by Jermyn and Isherwood[5] and adapted by Cronshaw, Myers and Preston[3] for the isolation of α-cellulose progressively removes the clay minerals, with a consequent progressive change in the X-ray diagram of the wall as reported and found confusing by the latter authors[2].

With these points in mind we have examined representative plants from most groups of the marine algae, using in the main the techniques of X-ray diffraction analysis and paper partition chromatography of hydrolysates of wall fractions, supplemented by electron microscopy and, very occasionally, polarization microscopy. The plants are dealt with in turn according to the nature of the crystalline wall substance present.

The X-ray diagrams of all the members of the Rhodophyceae we have examined, chosen to be representative of the group, are consistent with the presence of cellulose I as the skeletal substance, except in the Bangiales, a member of which will be discussed separately. The cellulose content of the wall and the degree of crystallinity of the cellulose are both rather low. The X-ray diagram is therefore revealed only after extensive removal of incrusting substances. For this removal, solutions of potassium hydroxide stronger than 10 per cent should not be used since with the red algae these solutions convert the cellulose I to cellulose II. The microfibrils contain a range of sugar residues other than glucose residues[2]; but it has recently been shown for one species at least that the central core of the microfibrils consists of cellulose I (ref. 6) alone. In all the Phaeophyceae we have examined, the X-ray diagrams again demonstrate clearly the presence of cellulose I as already reported for *Fucus*[7]. The clarity of these diagrams and the sharpness of the arcs distinguish them clearly from those of the red algae. Moreover, mercerization of the cellulose in the brown algae in potassium hydroxide solutions does not proceed at concentrations below 14 per cent. In the holocellulose of most of the Fucales examined an intense reflexion appears on the X-ray diagram corresponding to a spacing of about 14·1 Å. This indicates the presence of another crystalline wall substance,

though we are not certain whether this is originally present in untreated material or whether crystallization is induced by the treatment involved. Untreated material from stipes of *Alaria* (but not from the laminæ) undoubtedly contains a second crystalline component in addition to the cellulose.

The Chlorophyceae are, however, by no means so uniform as regards the nature of the skeletal substance, though cellulose I is again to be found in many families. There is no need here to document the presence of highly organized and well-crystalline cellulose I in the algae of Group 1, though some remarkable features of some plants in this group have only recently been pointed out[8]. Other members of the Chlorophyceae are also now known to contain this substance, including *Nitella*[9], *Ulva*[9] and *Ulothrix*, and perhaps *Spirogyra*[10], though there are peculiar features in this last species. The condition of the cellulose resembles that in either the Phaeophyceae or the Rhodophyceae, and all these algae should clearly be separated from those of Group 1.

The incrusting substances of the algal walls containing cellulose I as the crystalline component are constructed from uronic acids and a wide variety of sugars[3]. Among the sugars, galactose, arabinose and xylose predominate.

Beyond these, one plant in particular, namely, *Halicystis* (Siphonales) was long ago reported to contain cellulose II in place of cellulose I (ref. 11). Hydrolysates of the whole wall contain about equal quantities of glucose and xylose, and extraction of the wall with caustic soda solutions of increasing strength leaves unchanged both the X-ray diagram and the proportion of sugars in the hydrolysates of the residue[12]. The X-ray diagram contains arcs attributable to cellulose II together with a reflexion at about 13 Å. (12 Å. in our photographs) spacing which Roelofsen *et al.*[13], correctly in our view, refer to the large amount of xylan present. We have found that treatment of *Halicystis* holocellulose with caustic potash solutions of progressively increasing concentration causes a progressive decrease in the intensity of the arcs of long spacing, and that 4 *N* potassium hydroxide causes their final disappearance leaving the diagram of cellulose II. Hydrolysates of the very small residue (so-called α-cellulose) nevertheless still contain, besides glucose, about 40 per cent of xylose. Separation of the glucan and the xylan can, however, be achieved by treatment of the wall with cuprammonium. Most of the glucan dissolves and the precipitate gives an X-ray diagram of cellulose II. The residue insoluble in cuprammonium contains predominantly xylose residues and gives an X-ray diagram identical with that of the xylan to be discussed further on. We may compare the condition in *Halicystis* with that reported by Kreger[13] for *Hydrodictyon* (Chlorococcales), a freshwater alga. The walls of these plants contain glucan and mannan only, but neither of these yields an X-ray diagram until the wall has been treated chemically with, for example, dilute mineral acid. A composite diagram of both cellulose II and mannan is then obtained, symptomatic of a crystallization of glucan and mannan chains which, in the fresh wall, were at the most in a paracrystalline state.

Halicystis and *Hydrodictyon* are very special cases since only two sugars are used in the construction of the wall, and the boundary between crystalline and para- or non-crystalline phases must be vague. In these algae, in addition to glucose, mannose and xylose respectively must play an important part in both phases. Among the Siphonales there are other algae in which xylose or mannose form the only crystalline wall component and we now discuss these in turn.

Untreated walls of *Bryopsis*, *Caulerpa*, *Penicillus*, *Udotea* and *Halimeda* (all Siphonales) yield a remarkably distinct X-ray diagram which is quite different from that of cellulose and the spacings of which are listed in Table 1. Treatment of the material with boiling water, followed by chlorination and extraction with dilute alkali solution, leaves the diagram unchanged. In stronger alkali (10–18 per cent potassium hydroxide according to species) the wall dissolves. Suspensions of cell wall material of any of these species dried down to a thin film on glass show uniplanar orientation, that is, certain planes in the crystal lattice tend to lie parallel to the film (and therefore the cell wall) surface. The inner wall layers of the large filaments of *Penicillus dumetosus* give an X-ray diagram, illustrated in Fig. 1, which demonstrates that the crystallites in the wall lie parallel to filament length. *Penicillus* walls so treated as to remove incrusting substances (containing mainly glucose residues) yield, on hydrolysis, xylose together with a little glucose. The X-ray diagram (Fig. 1) is quite certainly not that of the β-1,4-linked xylan which can be isolated from the incrusting substances of cellulosic walls (cf. col. 1 and col. 2 in Table 1). The diagram does, however, resemble in character though not in detail those obtained from yeast glucan[14] and certain preparations of laminarin (Nicolai, E., unpublished results), both recognized β-1,3-linked glucans. Mackie and Percival[15] and Iriki *et al.*[16] have shown that the xylan of *Caulerpa*

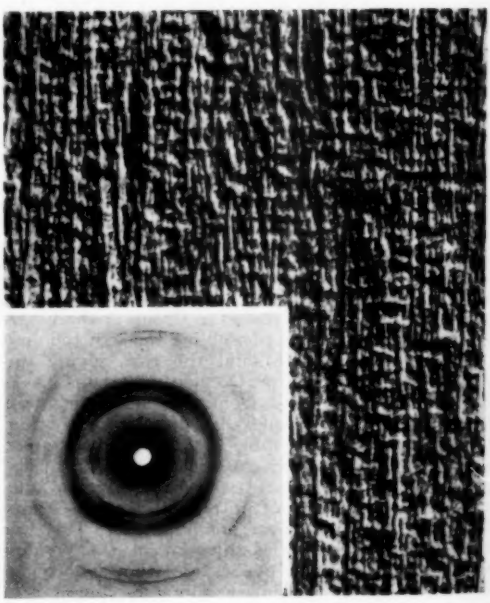

Fig. 1

Fig. 2

Fig. 1. X-ray diagram of a parallel bundle of inner wall layers of filaments of *Penicillus dumetosus*, filament length vertical. Copper K_α radiation

Fig. 2. Electron micrograph of wall lamella (untreated) from inner wall layer of a filament of *Penicillus dumetosus*, filament length vertical. (× 35,000)

Table 1. X-RAY SPACINGS OF XYLANS AND MANNANS FROM VARIOUS SOURCES

	Xylans		Mannans		
Elm*	*Penicillus*†	*Porphyra*† rhizoids	Ivory nut endosperm	*Dasycladus* (untreated)	*Porphyra*† 'cuticle'
	11·93 *V.S.*	11·90 *V.S.*			
8·15 *M.*					
			7·80 *W.*		
7·05 *M.*	6·80 *W.*	6·70 *M.*			
			6·61 *M.W.*	6·65 *W.*	6·58 *W.*
	6·12 *W.*				
		5·95 *V.W.*			
	5·50 *W.*		5·50 *V.S.*	5·51 *V.S.*	5·52 *V.S.*
5·42 *W.*					
	5·12 *M.*	5·22 *W.*			
			4·85 *M.W.*	4·84 *M.*	4·86 *M.W.*
4·57 *S.*					
	4·45 *S.*	4·46 *S.* (broad)	4·42 *S.*	4·39 *S.*	4·43 *S.*
	4·27 *S.*				
	4·01 *M.*		4·02 *W.*	4·03 *W.*	4·01 *W.*
3·94 *W.*					
			3·78 *M.S.*	3·74 *M.*	3·76 *M.*
3·51 *W.*	3·52 *W.*	3·52 *M.W.*	3·51 *M.S.*	3·47 *M.*	3·52 *M.*
	3·38 *W.*		3·29 *M.*	3·30 *W.*	3·30 *M.W.*
				3·21 *V.W.*	
	3·00 *W.*		3·04 *W.*	3·01 *W.*	3·03 *W.*
2·87 *W.*		2·89 *W.*	2·88 *M.*	2·89 *W.*	2·90 *W.*
	2·78 *W.*				
	2·72 W.		2·70 *M.W.*	2·68 *M.W.*	2·69 *M.W.*
		2·65 *W.*			
2·57 *M.*				2·54 *W.*	
	2·42 *W.*	2·48 *W.*			
	2·25 *V.W.*	2·30 *W.*		2·32 *W.*	2·32 *W.*
				2·13 *W.*	
				1·92 *V.W.*	

* Alcohol-precipitated from a 4 per cent potassium hydroxide extract of holocellulose.

† After extraction in boiling water followed by chlorination.

V.S., very strong; *S.*, strong; *M.S.*, medium strong; *M.*, medium; *M.W.*, medium weak; *W.*, weak; *V.W.*, very weak, reflexions.

is β-1,3-linked, and the latter authors have demonstrated the same linkage also in the xylans of *Bryopsis*, *Udotea*, *Halimeda* and some others. The X-ray diagram is therefore certainly that of β-1,3-linked xylan, and this is the crystalline component of the walls of these plants.

In the electron microscope, the cell walls of all these 'xylan weeds' are seen to be microfibrillar, illustrated here by an electron micrograph of an inner wall lamella of *Penicillus* filaments (Fig. 2). Of the two kinds of fibrillar bodies visible in Fig. 2, one, running approximately parallel to filament length (and about 100 Å. wide), resembles closely the microfibrils common in cellulosic walls. The other, less-abundant kind, lying approximately at right angles to the first, are not true microfibrils. The fibre diagram of inner layers (Fig. 1) clearly has its origin in the longitudinally oriented microfibrils. The birefringence of the wall, both in face view and in optical section, is nevertheless negative. This of necessity implies that the longitudinally oriented microfibrils are negatively birefringent, that is, the refractive index is maximum for light vibrating perpendicular to microfibril length. Both the X-ray diagram (Fig. 1) and this negative birefringence suggest that the molecular chains in these xylan microfibrils are arranged helically. Since the crystalline component of the walls of all the algae in this group is β-1,3-linked xylan then the same considerations must apply to all of them and, indeed, wall sections are always negatively birefringent. It is not therefore safe to carry over principles which apply to cellulose walls, by assuming that the major extinction position defines the microfibril direction, as has recently been done for *Bryopsis*[17]; though in *Bryopsis* itself the situation is complicated by the fact that extraction of the wall with alkali may reverse the sign of birefringence of the wall as seen in face view (but not, oddly enough, in optical section).

Since whole-wall hydrolysates of these walls contain xylose and glucose with little trace of other sugars, glucans are here prominent among the incrusting substances. These glucans are known to be also β-13,-linked[15,16].

On the other hand, as pointed out by Iriki and Miwa[18], wall hydrolysates of *Codium*, *Acetabularia* and *Halicoryne* (again all members of the Siphonales), treated successively with dilute solutions of sodium hydroxide, sulphuric acid and sodium chlorite, reveal mannose as the main constituent of the preparations. This substantiates our own independent findings on *Codium*, *Acetabularia*, *Dasycladus* and *Batophora* inasmuch as mannose is by far the most abundant sugar in hydrolysates of the whole walls though, in the water extract, galactose and glucose residues occur with the mannose residues. Glucose remains present in trace amounts throughout all stages of Jermyn and Isherwood's[5] extraction, including 24 per cent alkali. This sugar cannot therefore be a contaminant derived from starch, but must form a small but perhaps significant part of the wall organization. Walls of all these plants yield good X-ray diagrams of mannan (Table 1) except in *Codium*, where the reflexions are very diffuse unless incrusting substances have been removed. The inner parts of the relatively large central siphons of *Batophora* plants, which in face-view are strongly positively birefringent, yield an X-ray diagram which shows true orientation, indicating that the crystallites lie longitudinally in agreement with the birefringence. When examined with the X-ray beam parallel to the flattened faces of the wall and perpendicular to the length of the siphon, an even more beautiful diagram is obtained, as illustrated in Fig. 3. This is, so far as we know, the first oriented diagram of mannan to be pub-

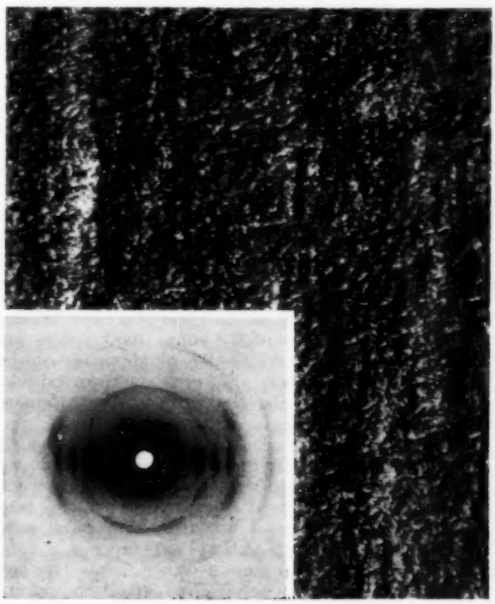

Fig. 3

Fig. 4

Fig. 3. X-ray diagram of walls of *Batophora oerstedi*; details as in Fig. 1

Fig. 4. Electron micrograph of wall of *Batophora oerstedi*; details as in Fig. 2. (× 47,000)

lished. The corresponding spacings are listed in Table 1 in comparison with those in the β-1,4-linked mannan of higher plants.

It is difficult to be certain of the form of the skeletal substance in these 'mannan weeds' as seen in the electron microscope, though the indications are that the mannan here consists of short fibrillar bodies of the order of 50 Å. in diameter (Fig. 4). In these walls, which contain no cellulose, we have been unable to find any true microfibrils such as are claimed to exist for the mannan in some higher plants[19]. This may be associated with short chain-length[18]. With all these mannan walls it is of interest to note that material treated with stronger alkali solutions (12–24 per cent potassium hydroxide) yield an X-ray diagram which is not that of the original mannan.

Now that it is established that xylan and mannan can individually form both the major fraction and the only crystalline component of cell walls, we have found it possible to resolve the condition in the red alga *Porphyra* (Bangiales) which has puzzled us for some time. The X-ray diagram of whole fronds of this plant (after chemical pre-treatment) is substantially that of mannan but shows additional reflexions of long spacing. This diagram turns out to be a composite one derived from both the cell walls proper and from a continuous surface sheath, a 'cuticle'. When cell walls alone are examined (as they may be *inter alia* with the rhizoids) the X-ray diagram is the same as those of the group discussed above in which the skeletal material is β-1,3-linked xylan. The corresponding spacings, listed in Table 1, are again unchanged after extraction of the material by boiling water, by chlorination and dilute alkali. The 'cuticle', on the other hand, yields an X-ray diagram of mannan. Both these diagrams conform with the fact that, after extraction of incrusting substances (which contain a range of sugar residues[3]) the cell walls hydrolyse to give xylose and the 'cuticle' to give mannose, each with small amounts of glucose. Cell wall hydrolysates contain no mannose and 'cuticle' hydrolysates no xylose. When chemically extracted cell walls are treated in a homogenizer, the homogenate can be seen in the electron microscope to contain a network of microfibrils indistinguishable from those found in, for example, the primary cell walls of higher plants. 'Cuticles' prepared in the same way show a granular structure. As in the mannan walls, the X-ray diagram of the 'cuticle' after treatment with stronger alkali solutions is not that of the original mannan.

Thus with *Porphyra* we find the odd circumstance that in different parts of the same plant xylose and mannose are used individually in the construction of the skeletal substance. For once the term 'cuticle', used loosely to describe the surface layer of many algae, has real meaning.

Cellulose no longer occupies a unique place as the skeletal substance in cell wall constitution as it was at one time thought to do. Even in the algae considered here, few indeed compared with the large groups which still await attention, we meet some forms in which the sole skeletal substance is a xylan and others in which mannan either forms the only crystalline material or (in *Hydrodictyon*) co-operates with a glucan in a wall which can be defined neither as a cellulose nor a mannan wall. These may be put alongside the greater number of algae in the walls of which the microfibrils, while certainly to be called cellulosic, nevertheless contain high proportions of xylan and the extreme case of the algae in Group 1 of Nicolai and Preston[1] in which the cellulose is pure. In all these forms, without any exception so far, some glucose at least is present in the skeletal material. Both cellulose and mannan are β-1,4-linked as is also xylan, according to X-ray evidence, when it is present in a cellulosic wall. The only skeletal xylan so far found, however, is β-1,3-linked, and it would seem to be significant that the glucans associated with it are also β-1,3-linked[15,16]. The situation in *Halicystis* appears to be an exception.

There is still some question whether the non-cellulosic skeletal substances form true microfibrils in the sense of the individual, easily separable bodies formed by cellulose. Even with the 'xylan weeds', what appear to be microfibrils cannot be separated, and torn edges of the wall never show the fringe of loose microfibrils typical of cellulose walls. This suggests that xylan microfibrils are interlinked. In the 'mannan weeds', separation of the wall into skeletal and incrusting substances seems pointless at the moment since here no microfibrils have yet been found. This fact, together with the observation that the walls of 'mannan weeds' are still coherent after treatment with strong alkali which leaves the only crystalline material in the form of granules, suggests that the role of microfibrils in strengthening a wall may have been over-emphasized.

The relative amount of skeletal material found in algal cells walls is in this respect not without interest. In the algae of Group 1, in which the skeletal material is pure cellulose, the microfibrillar component makes up between 30 and 75 per cent of the wall weight, a range which covers also most of the (cellulosic) secondary walls of higher plants. A somewhat similar figure holds also for the 'xylan weeds' ($\geq$ 50 per cent). With 'mannan weeds' the walls of which contain up to 90 per cent of mannose residues, no figure can be given on account of the difficulty of defining the skeletal component. In the remainder of the algae so far investigated, in which the microfibrils probably consist of a cellulose core with a hemicellulose cortex[6], the relative amount of skeletal material is low (2–20 per cent). All these walls are equally coherent whatever the proportion of the skeletal material.

Nicolai and Preston[1] made no mistake in concluding that among the Chlorophyceae variations occur in the construction of cell walls, though the basis on which their conclusions were founded was not completely reliable. Our newer observations show that while the bulk of the algae contain cellulose I, including most of those which Nicolai and Preston placed in Group 2, some make use for the same purpose of long-chain polymers built from sugars other than glucose. It is surely significant that this latter condition is almost exclusively confined to the Chlorophyceae. No plant so far investigated, with the exception of one (dubious) case, *Halicystis*, has been found to contain cellulose II. The algae of Group 1 still stand as unique among plants; the attempt to classify the rest of the algae into two groups must, however, be abandoned, and no new grouping is justified at this stage, particularly in view of complex forms like *Halicystis* and *Porphyra*.

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PRODUCTION AND PROPERTIES OF DI-FLUOROCARBENE

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THE di-fluorocarbene molecule, CF_2, was first detected spectroscopically in 1950, when its emission[1] and absorption[2] spectra in the ultra-violet region were photographed following the decomposition of fluorocarbon vapours in an electrical discharge. The observation that, provided the containing vessel were large enough, the absorption spectrum could still be detected seconds after the discharge was discontinued[2] was striking; it was inferred that CF_2 was a remarkably stable species, and that it disappeared after diffusion to the wall of the vessel.

Since 1950, CF_2 has been produced (and detected spectroscopically) by such a variety of methods that it is now apparent that its formation must be a favoured process. Table 1 lists some parent molecules which have been used and the methods of decomposition adopted. A zero order for the rate of disappearance of CF_2 produced in flash photolysis has been reported, and its long life-time confirmed[3].

Table 1

Parent molecule	Method	Reference
Fluoro-carbons	Elec. discharge	1, 2
CF_4	Furnace	4
CF_3COCF_3	Flash photolysis	5
CF_2Br_2	,, ,,	6
CF_2HBr	,, ,,	7
$CF_2ClCOCF_2Cl$	,, ,,	3
$CF_2{:}CF_2$	,, ,,	8
CF_2COOH	,, ,,	8

It has been suggested[8] that the production of CF_2 in the flash photolysis of hexa-fluoroacetone might follow the disproportionation:

$$2CF_3 \rightarrow CF_4 + CF_2$$

However, when the photolysis of CF_3I is used as a source of CF_2 radicals, no CF_4 can be detected among the volatile reaction products although there is an abundance of C_2F_6 (ref. 10). Further, although flash photolysis of CF_2Br_2 and CF_2HBr in the quartz ultra-violet region produces a high yield of CF_2, no CF_2 absorption could be detected following the flash photolysis of CF_3I (ref. 11) and CF_3Br (ref. 8) under the same conditions. Indeed, only when the latter molecules were photolysed in a specially constructed flash discharge cell which was transparent to wavelengths well below 2000 Å. (see Fig. 1) was it possible to observe a faint absorption spectrum of CF_2. It follows that a far greater energy input is required to obtain CF_2 from CF_3Br than from CF_2Br_2 or CF_2HBr, although the C—Br bond dissociation energies in the molecules would not differ very greatly. If the CF_2 production occurs through the disproportionation of substituted methyl radicals, then it is hard to understand why the disproportionation of CF_2Br and CF_2H should be so favoured as compared with CF_3.

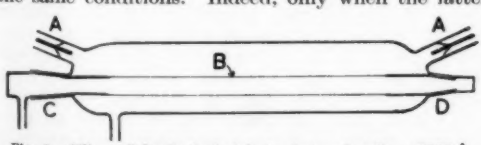

Fig. 1. Silica cell for flash photolysis at wave-lengths < 2000 Å. (Drawing not to scale.) *A*, Electrodes; *B*, thin silica (thickness 0·5 mm.); *C*, socket and cone, *B*.19; *D*, socket and cone, *B*.14

Finally, we have found that if CF_2Br_2 is flashed (under conventional conditions) in the gas phase in the presence of a large excess of oxygen, very few bromine atoms are produced, since the expected absorption spectrum of the BrO radical is barely detectable. (Flash photolysis of CCl_2Br_2 under the same conditions resulted in a very intense transient spectrum of BrO.) In particular, the yield of CF_2 is virtually independent of the oxygen pressure, which must exclude CF_2Br radicals as the precursors to CF_2 formation.

The data presented are all consistent with the primary production of CF_2 in a molecular split, as:

$$CF_2Br_2 + h\nu \rightarrow CF_2 + Br_2$$
$$CF_2HBr + h\nu \rightarrow CF_2 + HBr$$
$$CF_3COCF_3 + h\nu \rightarrow CF_2 + CF_3COF$$

and so on. A similar primary decomposition has been proposed in the xenon photosensitized decomposition of CF_4 (ref. 12). Such modes of decomposition are not in conflict with the suggestion that CF_2 is a relatively stable species having the properties of a two-electron deficient molecule rather than a di-radical. This hypothesis is favoured by: (1) the observation of an intense absorption spectrum of CF_2 following flash photolysis of tetra-fluoroethylene in the gas phase[8], which demonstrates the relative weakness of the carbon–carbon bonding in the olefine; (2) the lack of reactivity of CF_2 towards molecular oxygen, and (3) its zero-order rate of decay, demonstrating its lack of reactivity to other molecules of the same kind.

The difficulty in forming CF_2 following photolysis of CF_3Br is understandable in view of the energetics of the decomposition:

$$CF_3Br \rightarrow CF_2 + BrF$$

Such difficulty would not be anticipated in the cases of CF_2Br_2 and CF_2HBr, since in the first case both the bonds broken are weak, and in the second there is a considerable energy 'rebate' in the formation of the HBr molecule.

The ready decomposition of tetra-fluoroethylene into two CF_2 molecules, taken together with the other experimental results presented, suggests that in the fluorocarbon series it is not unreasonable to regard di-fluorocarbene as the simplest of the olefines. The splitting out of CF_2 from the substituted methanes and ketones listed is then analogous to the production of ethylene from ethyl iodide[13], and the Norrish Type II split in ketones containing hydrogen. Indeed, it has been proposed very recently[14] that the primary steps in the pyrolysis of hexa-fluoroacetone include the molecular decomposition:

$$CF_3COCF_3 \rightarrow CF_2 + CF_3COF$$

as well as the radical decomposition:

$$CF_3COCF_3 \rightarrow 2CF_3 + CO$$

The striking stability and the lack of chemical reactivity in di-fluorocarbene may be attributed, no doubt, to the influence of the strongly electronegative fluorine atoms. These would tend to attract the two electrons residing in the most weakly bound of the molecular orbitals, away from the neighbourhood of the carbon atom, on which they would otherwise be localized. As a result, the possibility of electron exchange interaction with other species would be much reduced, since there would be less possibility of electron overlap. This effect would not be anticipated in other di-halogenocarbenes, such as CCl_2 and CBr_2, since the electronegativities of Cl and Br atoms are not so very different from that of carbon. On Pauling's electronegativity scale, F = 4·0, Cl = 3·0, Br = 2·8 and C = 2·5. Indeed, attempts to prepare CCl_2 and CBr_2 photochemically from parent molecules analogous with those used for CF_2 production have so far proved unsuccessful[7].

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ACTION OF RADIATION ON SYNTHESIS OF DEOXYRIBONUCLEIC ACID IN NORMAL AND TUMOUR STRAIN CELLS

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HOWARD and Pelc[1,2] first showed that relatively small doses of radiation (50–200 r.) given during the deoxyribonucleic acid (DNA) pre-synthetic period, delayed the entry of cells into DNA synthesis, whereas cells already in DNA synthesis at the time of irradiation were unaffected by the doses given. These authors used phosphorus-32 uptake into DNA of bean root cells, and examined by autoradiography the number of cells synthesizing DNA over short periods up to 24 hr. during the interphase following irradiation. Holmes and Mee[3] and Kelly *et al.*[4], using regenerating liver and uptake of phosphorus-32 into DNA, again found that radiation doses of about 300 r. given during the pre-synthetic period delayed the onset of DNA synthesis: much larger doses (~2,000 r.) were required to depress DNA synthesis once it had commenced.

Lajtha *et al.*[5] found that doses of ~ 200 r. given to human bone marrow cells in culture during the pre-synthetic period delayed the entry of cells into DNA synthesis: the incorporation of ^{14}C-formate into DNA was observed by autoradiographic methods after various fractions of the interphase period following irradiation. On the other hand, it was found that a dose of 2,000–5,000 r. was required to reduce the rate of DNA synthesis to half, in cells which were already in synthesis.

In considering the effects of radiation on solid tumours *in vivo*, it is difficult to dissociate direct effects on the tumour from indirect effects acting through the surrounding host tissues[6]. In addition one such tumour usually represents a great variety of cell types; one way out of this difficulty is to use a tumour strain growing *in vitro*, and another possibility is to use an ascites tumour.

Painter and Robertson[7] used HeLa carcinoma strain cells in culture in conjunction with thymidine uptake and autoradiography, and found an increase in the number of cells in DNA synthesis at times up to 8 hr. after 500 r. of X-irradiation, and a decrease in the number of synthesizing cells after 17 hr. These results could partly be explained by inhibition of mitosis; first, an increase by retention in synthesis of cells which would normally have divided and, later, a decrease from the consequent depletion of mitosis; first, an increase by retention in synthesis Robertson pointed out, the results are certainly inconsistent with a delay in onset of DNA synthesis induced by radiation in the pre-synthetic period[7]. Again, using HeLa cells with thymidine and autoradiography, Yamada and Puck[8] found that radiation doses of 34–135 rads produced mitotic inhibition, but not a delay in the onset of DNA synthesis in these cells; this was in contrast with their findings with normal human cells in culture which showed a pre-synthetic delay in addition to inhibition of mitosis.

Dickson, Paul and Davidson[9] used *L* mouse strain cells in culture in conjunction with incorporation of ^{14}C–formate into DNA. These authors concluded that the effect of X-irradiation (800 r.) was to inhibit

division: the total amounts of cell constituents were found to be almost the same in the irradiated cultures as in the controls, and no alteration of ^{14}C–formate incorporation into DNA bases could be detected at times up to 24 hr. (more than one interphase period) after a dose of 800 r. to the cultures.

Kelly *et al.*[10] irradiated Ehrlich ascites tumour cells with a dose of 800 r. and investigated subsequent DNA synthesis by uptake of phosphorus-32 into DNA, and also by Feulgen staining combined with photometry. These authors found that the uptake of phosphorus-32 into DNA remained unaffected until 20 hr. after irradiation, at which time the average DNA content per cell was found to have increased; after 20 hr., a decrease in uptake of phosphorus-32 was observed, because, according to these authors, the cells were prevented from going through mitosis by the irradiation dose given.

The purpose of this article is to point out that those experiments which have shown inhibition of the onset of DNA synthesis with radiation doses of the order of a few hundred rœntgens in the pre-synthetic period have used cells freshly prepared from normal tissues, whereas those experiments using similar radiation doses which have failed to show an effect on the onset of DNA synthesis in the pre-synthetic period have used tumour-strain cells.

In order to explain their radiation results, it has been suggested by Lajtha *et al.*[5] that in human bone marrow cells there exists a 'trigger mechanism' connected with the onset of DNA synthesis, which is more sensitive to radiation than the process of DNA synthesis itself. Because of the results reviewed above, it would seem that this applies to the normal cells but not to the tumour strains.

It was reported some years ago that an increase in ultra-violet absorption by the cytoplasm occurred in irradiated cells[11]. More recently it has been shown[12–14] that an accumulation of soluble deoxy-derivatives, including deoxyribonucleoside triphosphates, occurred in irradiated thymus; these results were interpreted as indicating that inhibition of DNA synthesis by radiation was not caused by lack of precursors, but by failure of polymerization or breakdown in energy-generating mechanisms in the nucleus[14]. In this connexion, Creasey and Stocken[15] found that nuclear phosphorylation of purine and pyrimidine monophosphates to triphosphates is completely inhibited by 100 r. and reduced by 50–80 per cent after 25 r. In addition, in regenerating liver an increase in thymidine kinase and DNA polymerase is stopped by irradiation in the early pre-synthetic period, but not by irradiation in the DNA synthetic period[16].

Recently it has been shown by me[17] that, in three types of freshly prepared normal cells grown in culture (monkey kidney, embryo human, embryo mouse), the processes of DNA and nuclear protein synthesis are initiated together after the same waiting time (the pre-synthetic period) has elapsed, and thereafter the syntheses proceed approximately together. However, in the continuous tumour strains HeLa and *L* mouse, it was found that nuclear protein synthesis proceeds continuously during interphase and its onset does not await the onset of DNA synthesis, which commences after a waiting time has elapsed as in other types of cells[17]. Supporting evidence for this time-disconnexion of nuclear protein and DNA synthesis in tumour strains comes from the distributions of DNA and dry mass values found in Krebs ascites tumour cells[18]. Because of the genetic role at present ascribed to DNA, I have suggested[17] that the difference in timing of the onset of nuclear protein synthesis reflects a fundamental difference in metabolism between normal and tumour strain cells. It would appear from the radiation work reviewed here[1–10], that the processes responsible for initiation of DNA synthesis in these cells are also different in character.

Allfrey *et al.*[19,20] found that, in isolated calf thymus nuclei, the incorporation of amino-acid into protein required the presence of DNA, and that one role of DNA was in the production of adenosine triphosphate. Inhibitors of ATP synthesis were found to inhibit incorporation of amino-acid[20], and also the addition of histones to the nuclei was found to inhibit strongly respiration and nuclear phosphorylation[21]. Davison and Butler[22] and Vendrely *et al.*[23] reported that the total amount of all the basic groups in the nucleo-histone is approximately equal to the total amount of phosphate in the DNA.

It may therefore be permissible to suggest here that the simultaneous initiation of DNA and nuclear protein syntheses in normal cells[17] might reflect a mutual control system which would operate by regulating nuclear phosphorylation processes. The time-disconnexion of DNA and nuclear protein syntheses found in tumour strains[17,18] would imply dislocation of control of phosphorylation and would be consistent with the lack of radiosensitivity found in the DNA pre-synthetic period with these cells[7–10]. It is worth noting that Paul *et al.*[24] were unable to find evidence of any consistent radiation-induced depression of thymidine incorporation into thymidine triphosphate in either *L* mouse strain cells or in a lymphosarcoma cell strain, in contrast with the radiosensitivity of phosphorylation processes found in thymus nuclei[15].

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SUBSTRATE SPECIFICITY AND INHIBITION OF NICOTINAMIDE MONONUCLEOTIDE–ADENYLYL TRANSFERASE OF LIVER NUCLEI: POSSIBLE MECHANISM OF EFFECT OF 6-MERCAPTOPURINE ON TUMOUR GROWTH

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SYNTHESIS of nicotinamide adenine dinucleotides (coenzymes I and II) in most animal tissues involves formation of dinucleotide in the cell nucleus[1,2] catalysed by nicotinamide mononucleotide–adenylyl transferase (formerly known as coenzyme I - pyrophosphorylase)[3], thus:

nicotinamide mononucleotide + adenosine triphosphate ⇌ nicotinamide adenine dinucleotide + pyrophosphate

The biological significance of this reaction, which was discovered by Kornberg[4], has been discussed by Branster and Morton[3], and by Morton[5]. The work described here was carried out with enzyme obtained from isolated pig-liver nuclei[6]; either purified (specific activity approx. 3) or partly purified enzyme (specific activity, 0·7–1 μmole of nicotinamide adenine dinucleotide formed/min./mgm. of protein at 37° C., *p*H 7·6) was used.

Substrate specificity. In work on dinucleotide synthesis, the reaction mixture (0·2 ml., *p*H 7·6) contained approximately 0·005 unit[6] of enzyme, 25 μmoles of glycylglycine, 3·7 μmoles of magnesium chloride, 1 μmole of adenosine triphosphate (Sigma Chemical Co., U.S.A.; for purity, see ref. 7) and approximately 1 μmole of nicotinamide mononucleotide[6]; analogues replaced adenosine triphosphate or nicotinamide mononucleotide, as appropriate. In work on pyrophosphorolysis of dinucleotides, the reaction mixture (0·2 ml., *p*H 7·6) contained similar amounts of enzyme, buffer and magnesium salts, 20 μmoles of pyrophosphate and 1 μmole of nicotinamide adenine dinucleotide or of appropriate analogue. After incubation for 2½ hr. at 37° C., an appropriate portion (0·02–0·05 ml.) of the reaction mixture was applied to strips of filter paper (Whatman, 3 *MM*); nucleotides were separated by electrophoresis in 0·04 *M* sodium citrate, *p*H 4·2 for 3½ hr. at 400 V. (approximately 14 V. per cm.) and detected by printing on reflex document paper with ultra-violet light[7]. Quaternary nicotinamide compounds were detected by fluorescence after exposure to an atmosphere of methylethylketone–ammonia[8].

Table 1 shows the reactions investigated and the results obtained. It was previously shown[9] that the α-anomer of nicotinamide mononucleotide is not a substrate of the enzyme, which is almost certainly specific towards the β-*N*-glycoside bond. The pyridine mononucleotide (Table 1, reaction 7) probably contained both α- and β-anomers, but the proportions of these is not known; although unlikely, it is possible that the absence of a detectable reaction with this compound is due to a predominance of the α-anomer.

A number of purine riboside triphosphates, in addition to adenosine triphosphate, act as nucleotidylyl donors to nicotinamide mononucleotide (Table 1). Of the dinucleotides so formed, only nicotinamide hypoxanthine dinucleotide has previously been described[11]. Nicotinamide guanine dinucleotide was separated from the reaction products (Table 1, reaction 9) by chromatography on 'Dowex-2' formate with a gradient of formic acid; the dinucleotide was eluted between 1 and 1·5 *N* formic acid. The properties of the dinucleotide are shown in Table 2.

Table 1 shows that no enzymatic reaction could be detected with dinucleotides containing analogues of both nicotinamide and of adenine (such as 3-acetylpyridine hypoxanthine dinucleotide). With enzyme preparations obtained from acetone powders of pig-

Table 1. Substrate Specificity of Nicotinamide Mononucleotide–Adenylyl Transferase

Solid arrows indicate the reactions established. All reactions were carried out at *p*H 7·6 and 37° C., in the presence of magnesium chloride

1.	Nicotinamide mononucleotide	+ adenosine triphosphate	⇌ nicotinamide adenine dinucleotide	+ pyrophosphate*
2.	Nicotinic acid mononucleotide*	+ adenosine triphosphate	⇌ nicotinic acid adenine dinucleotide	+ pyrophosphate*
3.	Dihydro-nicotinamide mononucleotide	+ adenosine triphosphate	⇌ dihydro-nicotinamide adenine dinucleotide	+ pyrophosphate*
4.	Thio-nicotinamide mononucleotide†	+ adenosine triphosphate	⇌ thio-nicotinamide adenine dinucleotide††	+ pyrophosphate*
5.	3-Aldehyde-pyridine mononucleotide	+ adenosine triphosphate	← 3-aldehyde-pyridine adenine dinucleotide††	+ pyrophosphate*
6.	3-Acetyl-pyridine mononucleotide	+ adenosine triphosphate	← 3-acetyl-pyridine adenine dinucleotide††	+ pyrophosphate*
7.	Pyridine mononucleotide†	+ adenosine triphosphate	→ (no reaction)	
8.	Nicotinamide mononucleotide	+ hypoxanthosine triphosphate**	⇌ nicotinamide hypoxanthine dinucleotide††	+ pyrophosphate
9.	Nicotinamide mononucleotide	+ guanosine triphosphate**	→ nicotinamide guanine dinucleotide	+ pyrophosphate
10.	Nicotinamide mononucleotide	+ 8-azaguanosine triphosphate***	→ nicotinamide 8-azaguanine dinucleotide	+ pyrophosphate
11.	Nicotinamide mononucleotide	+ 6-mercaptopurine riboside triphosphate†††	→ nicotinamide 6-mercaptopurine dinucleotide	+ pyrophosphate
12.	Nicotinamide mononucleotide	+ *deoxy*-adenosine triphosphate**	→ (*deoxy*)-nicotinamide adenine dinucleotide	+ pyrophosphate
13.	Nicotinamide mononucleotide	+ uridine triphosphate**	→ (no reaction)	
14.	Nicotinamide mononucleotide	+ cytidine triphosphate**	→ (no reaction)	
15.		(no reaction)	← 3-aldehyde-pyridine hypoxanthine dinucleotide††	+ pyrophosphate
16.		(no reaction)	← 3-acetyl-pyridine hypoxanthine dinucleotide††	+ pyrophosphate

* Prepared according to Atkinson and Morton (ref. 10).
** Sigma Chemical Co., U.S.A.
*** We are grateful to Dr. R. B. Ross, National Institutes of Health, U.S.A., for a gift of this compound.
† Atkinson, M. R., Morton, R. K., and Naylor, R. (unpublished work).
†† Pabst Laboratories, U.S.A.
††† Prepared according to Roy *et al.* (ref. 17).

Table 2. COMPARATIVE PROPERTIES OF β-NICOTINAMIDE ADENINE DINUCLEOTIDE (COENZYME I) AND OF β-NICOTINAMIDE GUANINE DINUCLEOTIDE

	NAD*	NGD*
Ascending chromatography in isobutyric acid–water–conc. ammonia (33 : 66 : 1, v/v)	Single spot; R_F, 0·45	Single spot; R_F, 0·32
Treatment with methylethylketone–ammonia (ref. 8)	Fluorescent spot	Fluorescent spot
Bases liberated after hydrolysis and chromatography (ref. 12)	Adenine and nicotinic acid	Guanine and nicotinic acid
Absorption spectrum in 0·1 *N* HCl	$\lambda_{max.}$, 260 mμ (see ref. 13)	Similar to NAD, but inflexion near 270–280 mμ (cf. other guanine nucleotides; ref. 13)
Absorption spectrum in *M* KCN	$\lambda_{max.}$, 260 mμ and 325 mμ; E_{260} mμ/E_{325} mμ, 1·6	$\lambda_{max.}$, 266 mμ and 325 mμ; E_{266} mμ/E_{325} mμ, 1·96
Reaction with yeast alcohol dehydrogenase and ethanol at pH 10 in *tris* buffer*	K_m, $1·6 \times 10^{-4}$ *M*	K_m approx. 2×10^{-3} *M*; ($V_{max.}$ of reaction, 0·01–0·02 that of $V_{max.}$ with NAD)

* Abbreviations used: NAD, β-nicotinamide adenine dinucleotide; NGD, β-nicotinamide guanine dinucleotide; *tris*, 2-amino-2-hydroxymethylpropane-1 : 3-diol.

liver[4], Kornberg[4] earlier demonstrated reaction 3, and Preiss and Handler[14] reaction 2 of Table 1; Klenow and Anderson[15] previously demonstrated reaction 12 (Table 1) with an enzyme preparation from yeast[4].

Inhibition of synthesis of nicotinamide adenine dinucleotide. Kinetic work was carried out at *p*H 7·4–7·6; the reaction mixtures contained at appropriate concentrations, enzyme, glycylglycine, adenosine triphosphate, nicotinamide mononucleotide, magnesium chloride, and inhibitor. After incubation for 5 min. at 37° C., the reaction was stopped with trichloroacetic acid and the amount of nicotinamide adenine dinucleotide was estimated enzymically[6]. Determinations were made with several concentrations of inhibitor for each of two or more concentrations of the appropriate substrate (nicotinamide mononucleotide or adenosine triphosphate). Values of K_i (see Dixon and Webb[16], p. 22 *et seq.*) were estimated graphically. In studies with hypoxanthosine triphosphate (inosine triphosphate), the concentration of magnesium chloride was twice that of the total concentration of the nucleoside triphosphate present in each reaction mixture.

Values of K_m for several substrates were estimated similarly except that the rate of formation of nicotinic acid adenine dinucleotide was measured by estimation of pyrophosphate.

With hypoxanthosine triphosphate and nicotinic acid mononucleotide, inhibition of adenylyl transfer was competitive; Table 3 shows the K_i values for these compounds as inhibitors, and also the K_m values for these compounds when used as substrates for the enzyme.

Table 3. INHIBITOR CONSTANT (K_i) AND MICHAELIS CONSTANT (K_m) FOR SOME COMPETITIVE INHIBITORS OF SYNTHESIS OF NICOTINAMIDE ADENINE DINUCLEOTIDE (COENZYME I)

All values were determined at *p*H 7·4–7·6, and at 37° C.

Substrate*	Inhibitor	K_m (m*M*)	K_i† (m*M*)
1. Adenosine triphosphate		0·4	—
2. Hypoxanthosine triphosphate		1·8	—
3. Adenosine triphosphate	hypoxanthosine triphosphate	—	2·1
4. Nicotinamide mononucleotide		0·12	—
5. Nicotinic acid mononucleotide		0·4	—
6. Nicotinamide mononucleotide	nicotinic acid mononucleotide	—	0·38

* For 1–3, the co-substrate was nicotinamide mononucleotide; for 4–6, adenosine triphosphate.
† See Dixon and Webb (ref. 16, p. 22).

6-Mercaptopurine riboside (Sigma Chemical Co., U.S.A.) was converted to the corresponding 5′-monophosphate, which was treated with orthophosphate and dicyclohexylcarbodiimide to obtain the nucleoside triphosphate[17]. The nucleotide products were adsorbed on to charcoal, and then washed, eluted and dried *in vacuo* (preparation *A*). Preparation *A* contained components which appeared to be the 5′-mono-, di- and tri-phosphates of 6-mercaptopurine riboside, since they migrated similarly to hypoxanthosine-5′-mono-, di- and tri-phosphates on paper electrophoresis. Chromatography of preparation *A* on 'Ecteola' resin[17] gave four fractions, of which the last to be eluted (preparation *B*) was probably the 6-mercaptopurine riboside 5′-triphosphate; however, it was obtained in very low yield and in insufficient amount for chemical characterization.

Adenylyl transfer to nicotinamide mononucleotide was inhibited competitively by both preparations *A* and *B*. Separately, it was found that the 6-mercaptopurine itself, the riboside, and corresponding riboside 5′-phosphate do not inhibit adenylyl transfer at concentrations up to the concentration of the total 6-mercaptopurine nucleotide in preparation *A*.

With preparation *A* and nicotinamide mononucleotide as substrates, nicotinamide 6-mercaptopurine dinucleotide was formed (Table 1), confirming that preparation *A* contained 6-mercaptopurine riboside triphosphate.

From the kinetics of the competitive inhibition of adenylyl transfer by preparation *A*, and assuming that the only inhibitory material in preparation *A* was 6-mercaptopurine riboside triphosphate, K_i would be 0·3 m*M* if the yield of triphosphate were similar to that reported by Parks and co-workers[17]. Final K_i and K_m values for 6-mercaptopurine riboside triphosphate await preparation of larger amounts of material and adequate chemical characterization of the triphosphate.

Possible mechanism of the selective cytotoxic effect of 6-mercaptopurine against tumours. Branster and Morton[2] found that the activity per nucleus of the nicotinamide mononucleotide–adenylyl transferase of a number of tumours is considerably less than that of comparable normal tissues; the concentration of nicotinamide nucleotide coenzymes (coenzymes I and II) per nucleus (or per cell) is also considerably lower in tumours than in comparable normal tissues (see ref. 5). Morton[5] therefore proposed that inhibition of the nicotinamide mononucleotide–adenylyl transferase of the cell nucleus would lower the level of the nicotinamide coenzymes of tumour cells below that necessary for survival of the tumour, and indicated the general characteristics required for a physiologically effective inhibitor.

6-Mercaptopurine meets some of these criteria[5] for a compound selectively active against tumours. In susceptible cells, it is converted to 6-mercaptopurine riboside 5′-mono-, di- and tri-phosphates[18–20]. 6-Mercaptopurine riboside triphosphate is an analogue of adenosine triphosphate and, like hypoxanthosine triphosphate, is a substrate for nicotinamide mononucleotide–adenylyl transferase (Table 1). Although the chemical characterization of the components of preparation *A* is incomplete, the potent inhibition by preparation *A* and preparation *B* is almost certainly due to 6-mercaptopurine riboside triphosphate.

The selective toxicity of 6-mercaptopurine against tumour cells thus appears to be due to inhibition of

the biosynthesis of nicotinamide nucleotide coenzymes (coenzymes I and II); this inhibition occurs after the enzymic conversion of the purine to the purine riboside triphosphate, which competitively acts on the nicotinamide mononucleotide–adenylyl transferase of the tumour nucleus and effectively limits dinucleotide formation. Symptoms similar to those of nicotinamide deficiency have sometimes been observed in patients after prolonged treatment with 6-mercaptopurine[22].

Hypoxanthosine triphosphate is not sufficiently active to be an effective inhibitor of formation of coenzyme I *in vivo* (Table 3). Although nicotinic acid mononucleotide is strongly inhibitory (Table 3), the nicotinic acid adenine dinucleotide formed with substrate-levels of nicotinic acid mononucleotide may afterwards be converted to nicotinamide adenine dinucleotide. When nicotinic acid is administered to rats together with nicotinamide, it causes a marked decrease in the rate of formation of nicotinamide adenine dinucleotide in the liver, as compared with rats receiving nicotinamide alone. Table 3 shows that this effect is probably due to the inhibition of adenylyl transfer to nicotinamide mononucleotide by nicotinic acid mononucleotide.

6-Mercaptopurine was introduced by Hitchings *et al.*[23], as a potential antagonist of biosynthesis of nucleic acids, but recent findings[20,23] do not support this explanation of its action against certain human leukæmias and some transplanted tumours of animals. Although the evidence presented here shows that 6-mercaptopurine probably acts on tumours via the effect on biosynthesis of nicotinamide nucleotide coenzymes, since 6-mercaptopurine riboside triphosphate is an analogue of adenosine triphosphate, it could possibly influence a wide range of enzymatic reactions. The undesirable side-effects of 6-mercaptopurine may be partly associated with such multiple actions. Moreover, certain tumours have a limited enzymatic capacity for converting the purine to the nucleotide[20,24] and so escape the lethal depression of coenzyme synthesis. It thus seems preferable to inhibit formation of nicotinamide nucleotide coenzymes in tumours via analogues of nicotinamide mononucleotide, as previously suggested[5].

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MULTIPLE HÆMOGLOBINS AND TRANSFERRINS IN A MACAQUE SIBSHIP

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PHYSICO-CHEMICAL differences between embryonic, fœtal, and adult human hæmoglobins[1–4] and the occurrence of a number of transferrin phenotypes in man[5,6] have led several investigators to examine hæmoglobins and β-globulins in various Primates[7–15]. We report here on the hæmoglobins and β-globulins obtained from a related, inbred group of rhesus macaques (*Macaca mulatta*). Very young macaques have relatively high levels of alkali-resistant hæmoglobins, and the levels decrease as the animals grow older[9]. The number of β-globulins reported for macaques (*M. mulatta* and *M. irus*) has been large[11,12]. The results presented here are consistent with earlier findings on alkali-resistant hæmoglobins[8] and with reports of transferrin polymorphism[11–15] in various Primates. However, we

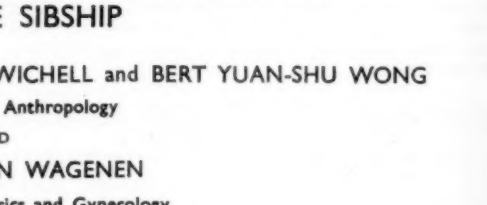

Fig. 1. Pedigree of inbred macaque sibship. Animal 1 died 14 days *post partum*; animals 7 and 8 were 90-day fœtuses taken by Cæsarean section. The symbols which do not contain numbers represent animals not sampled for this work

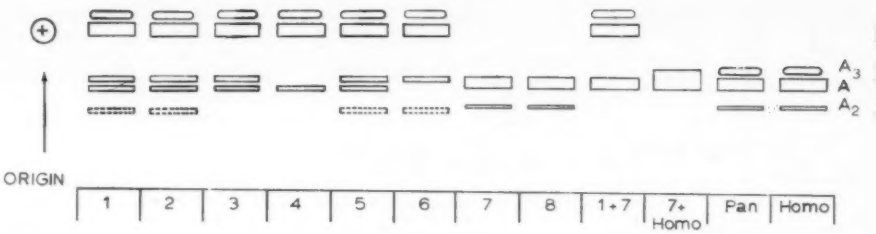

Fig. 2. Diagram of a starch-gel after electrophoresis showing multiple hæmoglobins from macaques. The human and chimpanzee (*Pan*) samples were run on this gel for comparison. *A*, A_3, and A_2 refer to the known hæmoglobins in this human sample and do not refer to the macaque or chimpanzee samples. When hæmoglobin from a fœtus (animal 7) was combined with either adult macaque or human hæmoglobin, the bands were incompletely resolved. The bands represented with dotted lines are those which stained weakly with benzidine

find this macaque sibship contains individuals that unequivocally display multiple hæmoglobins.

We received cord blood from two 90-day *M. mulatta* fœtuses taken by Cæsarean section, and from one of these we obtained blood from the aorta as well. Six animals related to the fœtuses in the same sibship were also sampled (Fig. 1). In this sibship the normal gestation period is 168 days[16]. Blood was drawn into tubes containing citric acid, sodium citrate, and dextrose. Serum and cells were separated by centrifugation, and hæmolysates were prepared by the method of Jonxis and Huisman[17]. Alkali-resistant hæmoglobins were demonstrated by the method of Singer *et al.*[18]. The hæmoglobin components were resolved on horizontal starch-gels, 13 per cent starch in *tris*-citrate buffer at *p*H 8·6. The gels were run for 16 hr. at 6 V./cm. with borate buffer, 0·3 *M*, *p*H 8·6, as the bridge solution[19]. Gels were sliced in half, one section was stained with a benzidine reagent (200 ml. of 50 per cent ethanol : 0·1 ml. of glacial acetic acid : 4 mgm. of benzidine ; 0·4 ml. of hydrogen peroxide added immediately before use). The other section of the gel was stained with bromphenol blue or amido-black. The β-globulins were resolved and identified on vertical starch-gels by a method previously described[14].

A diagram of a starch-gel electrophoretogram of the hæmoglobin samples is shown in Fig. 2. Three of the samples, including the two fœtuses, show two distinct components. In three of the samples there are 4 components, and two show 3 components ; four of these five samples show an additional component ; this was a very slow-moving band that stained weakly with benzidine. In order to obtain better resolution and a confirmation of these results, sample 1 was run for 24 hr. at 10 V./cm. The mobility of the principal component was 4–5 times that of human hæmoglobin *A* on the same gel. The several components seen in these animals have not been reported in other work on macaque hæmoglobins. Our previous experience and that of other investigators[20] with macaque hæmoglobins showed that only the single component reported by Sen *et al.*[6] appeared. There is one report that agar-gel electrophoresis demonstrated differences between the cord blood of a new-born macaque and its mother ; a second component was found in the cord blood of this new-born animal[7]. In addition, Lie-Injo *et al.*[10] and Tuttle *et al.*[9], using paper electrophoresis and veronal buffers, demonstrated other components in the hæmoglobins of macaques. They were able to resolve only 2 components in individual animals ; we can show as many as 5 components in one of our animals, and all the animals in this sibship have at least 2 hæmoglobins.

The transferrins of these animals are illustrated in Fig. 3. The diagram shows the bands which appeared on the autoradiographs of the starch-gels in which the sera were run. The half-gel which was stained with amido-black is illustrated in Fig. 4. It is clear to us that some of the bands in the β-globulin region on these gels are indeed transferrins. In the fœtal sera (animals 7 and 8) there are two distinct bands of protein in the β-region which stain faintly with amido-black but do not appear on autoradiographs. Their position corresponds to the 2–3 iron-binding bands in some of the adult sera. However, it was not possible to demonstrate iron-binding by these bands in the samples from the cord or the aorta of the two fœtuses. In general, we accept as transferrin only those bands which clearly take up radioactive iron-59. This criterion must be applied with care, since it is possible that these iron-binding proteins are saturated *in vivo* at the time the blood is drawn[21]. We have named the transferrin types according to the mobility of the iron-binding bands. Thus, a 1–1 or a 2–2 type is a macaque with only one such band, and a 1-1 type has the most mobile band ; a 2–3 type is an animal that has two iron-binding bands, the second and third in order of decreasing mobility.

Tables 1 and 2 summarize the results obtained for the 8 samples under discussion here. It is important to note that the proportion of alkali-resistant hæmo-

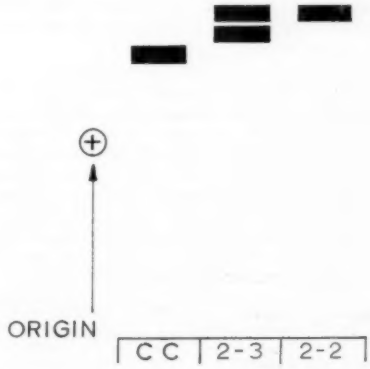

Fig. 3. Diagram of autoradiographs showing transferrin types of 8 animals in the macaque sibship. Three of the animals are type 2–2; 5, including the two fœtuses, are type 2–3. A human homozygous CC phenotype is shown for comparison

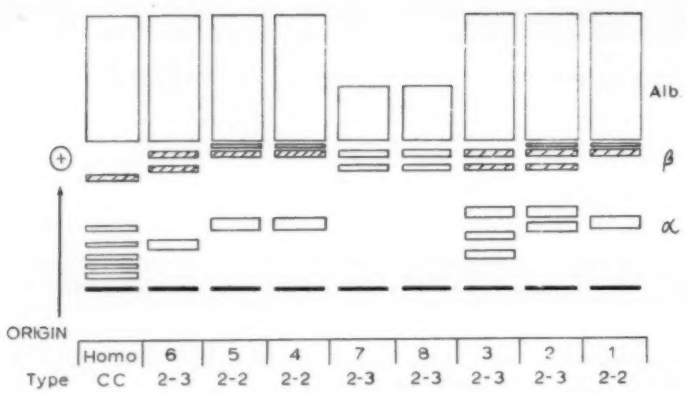

Fig. 4. Diagram of a starch-gel electrophoretogram showing those bands which stained with amido-black. Albumin, α- and β-globulin regions are noted. In the β-region, the hatched bands are those which show uptake of iron-59 on autoradiographs. A human sample, run on the same gel, is for comparison

globin is very high in the samples from the 90-day fœtuses. By the time an animal is one year old (animal 5) the level has dropped markedly. In 4 of the 5 adult animals we were unable to demonstrate any alkali-resistant pigment, and in the fifth the amount was negligible. The ultra-violet absorption spectrum of the alkali-resistant hæmoglobin from a fœtal sample (animal 7) was identical with published spectra of alkali-resistant fractions prepared from human hæmoglobin[10].

The hæmoglobin components seem to be inherited as simple autosomal alleles. The major problem here, as with human hæmoglobin A and A_2, is whether there are two loci involved or whether these hæmoglobins are allelic at the same locus. It seems clear that the principal component (that producing the densest stain and the largest spot) and one analogous to A_2 (human) will prove to be inherited as simple autosomal traits. The third component we definitely demonstrate may be analogous to human F, since it and the major band in macaque fœtuses correspond. In the adult animal, it is a slow-moving band. We found no alkali-resistant hæmoglobin in these adults, hence the analogy with human hæmoglobin may not hold. The samples taken from the two fœtuses were so small that only alkali-denaturation studies and electrophoresis on starch-gels were possible.

Table 1. Hæmoglobins and Transferrins

Animal	No. of hæmoglobins	Transferrin type
1	4 (5)	2-2
2	4 (5)	2-3
3	3 (4)	2-3
4	3	2-2
5	4 (5)	2-2
6	3 (4)	2-3
7	2	2-3 (?)
8	2	2-3 (?)

Table 2. Alkali-Resistant Hæmoglobin

Animal	Age	Sex	Alkali-resistant hæmoglobin (per cent)
1	Adult	♀	0
2	Adult	♀	1·2
3	2 years	♀	0
4	4 years	♂	0
5	1 year	♀	0
6	14 days	♂	50·0
7	Fœtus	♂	85·7 (cord)
8	Fœtus	♀	78·3 (cord)
			75·6 (aorta)

The nomenclature used for the transferrin types implies that the inheritance of these β-globulins is due to simple non-dominant alleles, as is the case for the human transferrins[5]. The pedigree (Fig. 1) bears this out, but samples from the missing animals are necessary to demonstrate this conclusively.

Sufficient data[11,12] have been reported so that it is reasonable to predict that transferrin polymorphism exhibited by macaques may be due to the action of natural selection on different populations of these animals. Unfortunately, it is not possible to work out the inheritance of many of these transferrins until various different phenotypes have been cross-bred. The number of hæmoglobins may not be explained so simply. Our experience with a large number of macaque samples from animals captured in three different localities (India, Malaya, and the Philippines) indicated that no more than two hæmoglobins or components existed. The results presented here on a group of related animals demonstrate that multiple hæmoglobins exist and imply that these hæmoglobins are inherited. We also have unpublished data indicating that multiple hæmoglobins among the Prosimian Primates is the rule.

A final statement about the mode of inheritance of these hæmoglobins cannot be made until additional data become available. This problem of multiple hæmoglobins in the non-human Primates is just coming under investigation. Thus, we are only starting on an evolutionary and genetic problem of great fascination.

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EFFECT OF DIETARY ASCORBIC ACID ON THE SILKWORM, *BOMBYX MORI*

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THERE are a few papers suggesting the importance of ascorbic acid in the nutrition of the silkworm, *Bombyx mori*. Gamo[1] showed that the content of ascorbic acid in fresh mulberry leaves changed in relation to conditions influencing the food value of leaves, and that the amount of ascorbic acid in the larval body is dependent on that in the leaves. According to Murthy[2], ascorbic acid was effective for increasing survival of larvæ. From these papers, however, it may still be impossible to draw a conclusion as to the precise role of ascorbic acid in silkworm nutrition. Ascorbic acid was added to the diet for pink bollworm[3], but most artificial diets developed for other phytophagous insects do not contain ascorbic acid. The failure to rear these insects on such deficient diets has not, so far, been mentioned. On the other hand, Thorsteinson[4] indicated the phagostimulant action of ascorbic acid for several phytophagous insects, and Dadd[5] showed that the inclusion of ascorbic acid in synthetic diet was necessary for normal development to the adult stage in two species of locusts. In locusts, however, ascorbic acid was proved to have negligible effect on the feeding, but was considered to play some part in the processes occurring at the moult. It has long been felt that the role of ascorbic acid in silkworm nutrition, as suggested by previous authors, must be confirmed. The results presented in this article are based largely on observations on feeding responses of silkworm larvæ given ascorbic acid and on its growth-promoting effect, when added to artificial diets for this insect.

For feeding tests an agar diet was used. Its composition was similar in principle to that previously used to test the effect of sugars on silkworm feeding[6]. The diet was composed of agar, starch, sucrose, soybean casein, mulberry leaf powder, and cellulose powder. Twenty ml. water was added to 2·5 gm. of the dry diets. Where necessary, 1 part of soybean oil was added to 12·5 parts of the dry diets, and 30 mgm. of ascorbic acid was contained in 20 ml. of water.

Table 1

	Larval distribution (per cent)
Disk without ascorbic acid	8·5 ± 1·5
Disk with ascorbic acid	83·0 ± 7·2
Filter paper	8·5 ± 7·9

(Five replicates)

Two techniques were used to test the effect of ascorbic acid on feeding behaviour: (1) a two-choice design in which newly hatched, unfed larvæ were allowed to choose the dietary media on which to feed, (previously used by Beck[7] for the European corn borer) and (2) counting the number of fæces produced by newly hatched larvæ. The agar media were cut into disks and placed side by side on the filter paper in a Petri dish. Approximately 25 larvæ were used for each dish and dishes were kept at 25° C. in the dark for a few hours until counts were made on larval distribution resulting from feeding movement. In Table 1 the result on larval distribution is presented. Most of the larvæ (83 per cent) settled on disks containing ascorbic acid, and the remaining small number of larvæ were either on disks without ascorbic acid or on the filter paper. When disks were placed some distance apart, the result was not necessarily clear cut. Later, the newly hatched larvæ, ten in a group, were allowed to feed on the agar diets for 18 hr. at 25° C. At the end of that time, the larvæ were removed from the diet and, with the aid of a magnifying glass, counts were made of the number of fæces. As shown in Table 2, ascorbic acid had a high stimulative action on feeding, irrespective of the presence or absence of added soybean oil, with a high statistical significance. Further, a slight phagostimulant effect of either soybean oil[8,9] or plant sterols[9] was overshadowed by the strong phagostimulant action of ascorbic acid.

Table 2

Ascorbic acid	Soybean oil	Fæces count per larva
−	−	4·3 ± 2·0
+	−	28·3 ± 4·8
−	+	9·4 ± 1·7
+	+	30·2 ± 4·1

(Four replicates)

Since it became evident that ascorbic acid was a strong phagostimulant for the silkworm, an experiment was carried out using artificial diets to assess the effect of dietary ascorbic acid on growth. The diets used for this purpose were not agar-based ones, but culture media solidified with potato starch, as previously used for nutritional work on this insect[8–10]. The following materials were included for basal media: mulberry leaf powder (10 per cent on a basis of dry matter), potato starch, glucose, soybean casein, soybean oil, salt mixture, soybean sterols (or β-sitosterol), vitamin B mixture, and cellulose powder. The amount of ascorbic acid to be added ranged from 0·2 to 40·0 mgm. per gm. dry diet. One set of results obtained is presented in Table 3, in which mean body-weight after a 20-day rearing period is shown. Even at a concentration of 0·2 mgm. ascorbic acid per gm. dry diet, good growth was obtained, and the growth was improved by increasing the amount of ascorbic acid in the diets. However, best growth and development were obtained with either 4·0 or 10·0 mgm. per gm. dry diet. Ascorbic acid was also very effective in decreasing the mortality at older larval stages.

Table 3

Ascorbic acid added (mgm./gm. dry diet)	Body-weight of individual after 20-day rearing (mean of 2 batches) (mgm.)
0	49·9
0·2	75·8
1·0	95·5
2·0	132·6
4·0	153·6
10·0	148·9
20·0	111·4
40·0	141·8

Thus ascorbic acid is necessary for better growth and development of the silkworm and should be added to artificial diets as an essential component, as in the locust[11]. The effects of ascorbic acid seem possibly to be due to its phagostimulant action. However, there is a possibility that the growth-promoting effect might be a combined one of those on feeding and nutrition. Further work is necessary on the physiology of ascorbic acid in the silkworm.

The details of this report will be published in the *Bulletin of the Sericultural Experiment Station*.

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VENTURICIDIN: A NEW ANTIFUNGAL ANTIBIOTIC OF POTENTIAL USE IN AGRICULTURE

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DURING 1957–58 we screened some 8,000 soil actinomycetes for ability to produce new antibiotics active against apple scab (*Venturia inaequalis*). Here we summarize work which terminated in the isolation and biological evaluation of a new antibiotic, 'venturicidin', now being investigated for use in agriculture.

Isolation from Soils

Actinomycetes were isolated by inoculating serial dilutions, from a suspension of soil in water, into agar media containing different carbon sources. After one week's incubation, the 'pin-head' colonies were transferred to potato-dextrose agar slopes in small cylindrical glass bottles.

The isolated strains were tested for antibiotic production in surface culture by removing 'plugs' from well-grown cultures on potato-dextrose agar and determining the inhibition zones produced when the agar 'plugs' were placed on microbiological assay plates seeded with homogenized mycelium of *V. inaequalis*. Strains producing inhibition zones were afterwards tested for antibiotic production in submerged culture on three different fermentation media by 'cup-plate' assay of the fermentation broth against *V. inaequalis*, on the 2nd, 3rd and 4th days after inoculation. Antibiotics giving zones of inhibition greater than 20 mm. diameter were again produced by fermentation, but this time on what had been found the best of the three submerged fermentation media; the antibiotic production was assayed by incorporating serial dilutions of homogenized broth into agar slopes and then inoculating the surface with an homogenized culture of *V. inaequalis*. Antibiotics that completely inhibited growth of *V. inaequalis* at broth dilutions in the range 1 in 80 to 1 in 1,280 were considered to be worth extraction.

The technique was to submit broths from completed fermentations to a number of different extraction procedures, with 'cup-plate' assay of extracts and spent broths. Similarly, adsorption or non-adsorption on ion-exchange resins was determined by 'cup-plate' assay of the resin filtrates and eluates. The results enabled a suitable procedure to be devised for preparing a crude extract of the antibiotic.

Fermentations with the selected strains were repeated under optimum conditions in submerged culture, but now in two 5-litre mechanically agitated and aerated laboratory fermenters[1]; the bulked broth at the end of the fermentation was extracted by the selected method to give approximately 25 ml. of a highly concentrated solution in acetone. These 'first concentrates' were scrutinized in several ways (extraction pattern, ultra-violet absorption spectrum, chromatography and bio-autographs, microbiological 'spectrum' *in vitro*, phytotoxicity and antifungal foliage protection tests) so as to eliminate unwanted antibiotics (polyenes, antimycin complex, oligomycin complex and cycloheximide). It became clear, after considerable experience of this procedure, that an apparently new antibiotic, designated venturicidin, was being produced by several strains of streptomyces and that it was both non-phytotoxic and highly active against a wide range of plant pathogenic fungi.

In general, it was observed that strains producing venturicidin gave 'first concentrates' which completely inhibited *V. inaequalis* in tube assay down to dilutions of 1 in 100,000. Similarly, the 'first concentrates' usually gave inhibition zones against *Cercospora melonis* in 'cup-plate' assay down to dilutions of the order of 1 in 100,000. This plant pathogenic fungus being more suitable than *V. inaequalis* for use in assays of this type, it was selected as convenient for microbiological assay of solutions of venturicidin in 50 per cent acetone.

'First concentrates' containing venturicidin were obtained from several strains and gave good protection in greenhouse tests against *V. inaequalis* when applied at dilutions of 1 in 500 to 1 in 2,000 to the foliage of pot-grown apple root stocks (Malling/Merton 106 and Malling XVI). Similarly, excellent protection was given to barley seedlings against *Erysiphe graminis*. In no test did venturicidin show any signs of phytotoxicity.

Further extraction tests on fermentation broths accompanied by appropriate 'cup-plate' assays led

to an improved technique for extraction; this was applied to the preparation in two 5-litre laboratory fermenters of an improved 'second concentrate' and then for provision of crystalline venturicidin from 225-litre stainless steel pilot-plant fermenters.

The method of preparing crystalline venturicidin is summarized here, together with its main chemical and biological properties.

Crystalline Venturicidin

Strains. Venturicidin is produced by three distinct strains of *Streptomyces* having a general resemblance to *Streptomyces griseolus* and *S. halstedii* and some affinity with *S. xanthophaeus*. They have been found in soils from many parts of England and also from New Zealand. Typical members of these three strains have been deposited in the National Collection of Industrial Bacteria under Culture Nos. N.C.I.B. 9198, N.C.I.B. 9199 and N.C.I.B. 9200.

Fermentation. Vegetative inoculum was grown in a 5-litre laboratory fermenter containing 'Sabouraud' and malt medium (2 per cent 'Oxoid' malt extract, 4 per cent glucose, 1 per cent 'Oxoid' peptone) and transferred at the 28th hr. to a 225-litre pilot-plant fermenter containing the medium (3 per cent Rickard's oat-flour, 1 per cent light precipitated chalk, 0·75 per cent glucose). The fermentation temperature was 28° C. and the broth was extracted after three days.

Extraction. The broth was filtered at *p*H 6 and the filtrate discarded. The mycelium was extracted at room temperature with acetone and the extract was freed from acetone by concentration under reduced pressure with addition of a little water, as necessary. The aqueous solution was extracted at neutrality with light petroleum (60–80°) to remove antifoam oil, and any venturicidin that had been inadvertently extracted was afterwards recoverd by re-extraction with 90 per cent methanol. The aqueous methanol solution was freed from methanol under reduced pressure, and the aqueous residue was combined with the main aqueous solution before extraction with ethyl acetate. The ethyl acetate extracts were dried over sodium sulphate and taken to dryness under reduced pressure to give an oily solid.

Crystallization. A typical crystallization was carried out by the following procedure. To 2·48 gm. of crude oily solid, 25 ml. of carbon tetrachloride were added. The oil dissolved, but precipitation of semi-crystalline material occurred immediately; it was removed by filtration and washed with carbon tetrachloride to give 610 mgm. of almost colourless solid. This was dissolved in 7 ml. of hot methanol, and 2 ml. of water were added. On cooling, 590 mgm. of needle-shaped crystals (m.p. 128–132° C.) separated.

Alumina chromatography. The procedure used in a typical purification was as follows. A column was prepared with 15 gm. of 'Woelm' alumina (neutral grade 1): 579 mgm. of crude oily solid were added in 5 ml of chloroform. Continued elution with chloroform eliminated 145 mgm. of oily material; after washing the column with ethyl acetate, 95 mgm. of a biologically active fraction were eluted with ethyl acetate containing 5 per cent methanol. This material was crystallized from methanol/water to give a solid (m.p. 135·5–137·5° C.) and further purified by alternate re-crystallizations from ethyl acetate/light petroleum and methanol/water, to give crystalline material [m.p. 140–142° C. (uncorrected), $(\alpha)_D$ = + 110° (*c.* 1 per cent, chloroform)], containing only one biologically active substance, as shown by bio-autographs of chromatograms developed in several solvent systems.

Chemical properties. Venturicidin is a neutral substance. It is soluble in butanol, ethyl acetate or chloroform. It is stable at room temperature at *p*H 7. It gives negative ferric chloride, ammoniacal silver nitrate and anthrone tests, but shows a red coloration with 2,4-dinitrophenylhydrazine.

Prof. B. Lythgoe, of the University of Leeds, is now investigating the structure of venturicidin, and we are grateful to him for the following results:

m.p. 142–143° C.; $[\alpha]_D$ = + 114° (*c.* 1 per cent, chloroform).
Found C 64·7, 65·1; H 9·0, 9·0, 8·95, 8·85; N 1·75, 1·75, 1·75; O (Unterzaucher) 23·95, 23·5.
Calculated for $C_{12}H_{11}O_{12}N$: C 65·0; H 9·0; N 1·75; O 24·2 per cent.

Venturicidin can be conveniently prepared as a 10 per cent water-miscible solution in 'ethyl cellosolve' with addition of a suitable surface-active agent; on dilution with water this provides solutions of venturicidin, at concentrations between 200 and 25 μgm./ml., suitable for application to plant foliage.

Biological activity of venturicidin in vitro. Crystalline venturicidin is antifungal but not antibacterial, though when crude it is sometimes associated with antibacterial material. Minimum concentrations in 50 per cent acetone producing inhibition zones in 'cup-plate' assays are given in Table 1.

Table 1. Minimum Concentrations of Venturicidin needed to give Inhibition Zones in 'Cup-plate' Assays against Plant Pathogenic Fungi

Fungus	Minimum inhibitory concentration (μgm./ml.)
Ascochyta pisi	40·0
Aspergillus niger	5·0
Alternaria tenuis	5·0
Botrytis cinerea	2·5
Cercospora melonis	1·25
Didymella lycopersici	2·5
Fusarium nivale	2·5
Glomerella cingulata	10·0
Helminthosporium avenae	2·5
Phoma betae	5·0
Pythium de Baryanum	5·0
Penicillium expansum	10·0
Thielaviopsis basicola	20·0
Trichothecium roseum	5·0
Ustilago maydis	5·0
Verticillium albo-atrum	2·5

Tube-dilution assay (antibiotic incorporated in agar and test fungus inoculated to the surface) gave the results as follows:

Venturia inaequalis: complete inhibition at 1 μgm./ml. and partial inhibition to 0·1 μgm./ml.
Sclerotinia fructigena: complete inhibition at 0·3 μgm./ml. and partial inhibition to 0·01 μgm./ml.
Botrytis cinerea: complete inhibition at 0·3 μgm./ml.
Glomerella cingulata, *Cercospora melonis*, *Fusarium nivale*: partial inhibition to 0·3 μgm./ml.

Venturicidin also exhibited exceptionally high activity against *Nocardia rubra* (complete inhibition by 0·08 μgm./ml. after 7 days in liquid tube dilution assay) and markedly inhibited *Aspergillus fumigatus* and *Blastomyces dermatitidis* at concentrations between 20 and 1·25 μgm./ml.

Greenhouse protection tests with venturicidin against plant pathogenic fungi. Crystalline venturicidin gave excellent protection against a number of plant pathogenic fungi when used as a prophylactic foliage spray in greenhouse tests. For example, at LD_{90-95}, 20 μgm./ml. venturicidin was approximately as effective as 200 μgm./ml. Captan against apple scab (*V. inaequalis*) and was about as good as 200 μgm./ml.

Karathane against barley mildew (*E. graminis*). Venturicidin is also exceptionally active against broad bean rust (Hunnam, D., and Smith, C. B. F., private communication) and has some activity at concentrations of 20–40 μgm./ml. against cucumber mildew (*Erysiphe cichoracearum*), apple mildew (*Podosphaera leucotricha*) and grey mould (*B. cinerea*) on tomatoes and vines. Venturicidin showed local systemic action in apple foliage and some eradicant action against *V. inaequalis*.

Pharmacology of venturicidin. Preliminary results on mice and dogs show venturicidin to be non-toxic orally, besides having low intraperitoneal toxicity and relatively low intravenous toxicity (Tomich, E. G., private communication).

It is concluded that venturicidin is a new antibiotic with considerable potential use in agriculture. It is now undergoing commercial evaluation as a fungicide against apple scab (*V. inaequalis*).

We thank our colleagues, Mr. W. K. Anslow and Mr. D. Fletcher, who were responsible for the 5-litre and pilot-plant fermentations.

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STRUCTURE OF THE PLANT STEM IN RELATION TO ADVENTITIOUS ROOTING

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THE ability of one-year-old stems to form adventitious roots has been found, in a range of fruit plants, to be closely related to the anatomical structure of the primary phloem of the stem. Shoots of shy-rooting varieties are frequently characterized, in this tissue, by a high degree of sclerification, that is, differentiation into fibres and sclereids. In Conference pear, for example, an almost continuous cylinder of mature thick-walled fibres without living protoplasts encircles the secondary phloem and appears, in transverse section, as a ring of lignified tissue (Fig. 1). In ready-rooting plants it is usual to find that most of the primary phloem parenchyma cells retain living protoplasts even when they are in a stage of transition to sclerotic parenchyma, or sclereids. It follows, therefore, that in stems possessing a high rooting propensity many of the phloem rays abut, at their distal ends, on areas of living tissue (Fig. 2) in contrast to the shy-rooters, where the phloem rays are frequently blocked by heavily lignified elements (Fig. 1). The degree of continuity of the sclerenchymatous ring is compared, in Table 1, with ease of propagation as shown by the behaviour of stooled plants reported by Garner[1]. As the propagation capacity falls, the continuity of the ring increases.

A similar general relationship between the degree of sclerification of the primary phloem and rooting ability has been observed among rootstocks for stone fruits, and in a range of flowering shrubs from temperate regions. Shoots of certain shy-rooting tropical plants, including *Hevea brasiliensis* sent from Malaya and *Acacia mearnsii* sent from Natal, have been found to possess, at the base of one-year-old stems, an almost unbroken cylinder of mature lignified elements, similar in continuity to that of the shy-rooting Conference pear, but differing in composition as regards type of element. Working with stem cuttings of the olive in Italy, Ciampi and Gellini[2] noted a similar connexion between the absence of a sclerenchymatous ring and rooting potential. Thus a relationship between structure and rooting capacity would appear to be applicable to a wide range of species. That the presence of periphloic sclerenchyma is not the sole cause of the prevention of easy rooting is, however, shown by the following examples: species of *Pittosporum* lacking such sclerenchyma are, nevertheless, difficult to root, as are also certain *Erica* spp. where a phellogen is laid down on the inner side of the periphloic sclerenchyma (C. R. Metcalfe, private communication).

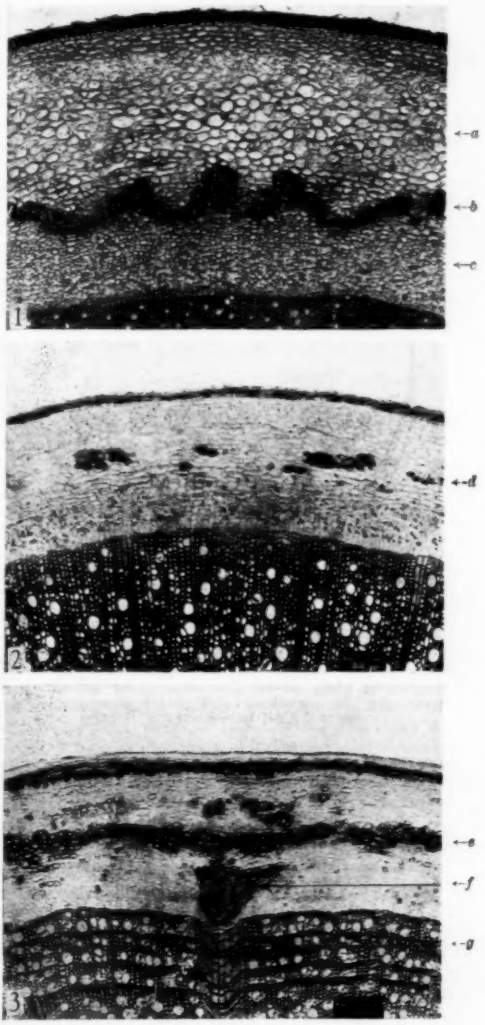
Inherent differences between clones in the degree of sclerification of the primary phloem have been found to be relatively stable in material grown under comparable conditions at East Malling. Environmental effects may, however, be superimposed on the inherent structure, differentiation of the primary phloem parenchyma cells into fibres and sclereids being retarded, for example, by shading, and by partial etiolation as in the earthing up of stool shoots, or marcotting of aerial stems.

Comparisons between shoots exhibiting juvenile characteristics and mature shoots, both derived from the same plant, have indicated that, in seedling apples, the basal juvenile shoots are relatively free from sclerotic cells in the primary phloem compared with lateral shoots from fruiting branches close to the apex of the maturing tree. These observations provide indirect evidence of a relationship between

Table 1. Relation between Propagation Capacity and Primary Phloem Structure in Stems

Variety	Propagation capacity	Continuity of fibre ring* Per cent	Mean
Apple rootstocks			
M.XIII		28	
M.V.	Excellent	43	41
M.XI		52	
M.IV		59	
M.XVI	Good	64	64
M.II		69	
M.XII		69	
M.XXV	Fair	71	75
M.VIII		85	
Crab C		82	
Northern Spy	Poor	84	84
Ivory's Double Vigour		86	
Scion varieties			
Apple			
Bramley's Seedling		86	
Cox's Orange Pippin	Poor	83	80
Lane's Prince Albert		70	
Pear			
Conference		99	
Williams's Bon Chrétien	Poor	96	98

* Proportion of radii through the primary phloem blocked by sclerenchyma.



Figs. 1-3. Structure of primary phloem. (1) Conference pear; (2) Malling V. apple rootstock; (3) *Fagus sylvatica* (beech) *a*, cortex; *b*, sclerenchymatous sheath; *c*, phloem; *d*, primary phloem fibres; *e*, sclerenchymatous sheath; *f*, sclerosed phloem ray; *g*, multiseriate xylem ray

anatomy and rooting potential since loss of rooting ability over the period of change from juvenility to maturity, first reported in 1929 by Gardner[3], is now well established for a number of plants, including the apple. Ready-rooting sphaeroblast shoots, artificially induced by continuous removal of normal bud meristems[4], have also been found to have fewer fibres and sclereids in the primary phloem than shy-rooting normal shoots from the same apple clone.

Sphaeroblasts are small woody inclusions occasionally found in the bark of many tree species. These nodules, which are separated from the central vascular cylinder, possess a cambium and may continue to grow over a period of years, forming annual rings and eventually protruding conspicuously from the trunk. Following certain stimuli, including severe pruning accompanied by the removal of all lateral buds and of any subsequent shoots from dormant buds, young sphaeroblasts may grow out producing either rosettes of leaves or long shoots. The work of Baldini and Mosse[7] on the inception of sphaeroblast primordia in apple has shown that sphaeroblast shoots arise adventitiously from a massed meristem originating in a primary tissue, thus providing anatomical evidence of juvenility, supporting the observations of Stoutemyer[5] and Wellensiek[6] that adventitious shoots from sphaeroblasts exhibit juvenile morphological characters. If sphaeroblast shoots are in a more juvenile phase than those arising normally from a cambial meristem, the differences in anatomy between them provide a further example of the increasing sclerification of the primary phloem with advancing maturity of the tissue and they are in line with the suggestion made by Foster[8] that sclerification is an aspect of tissue senescence.

It is obvious that if a correlation exists between the degree of sclerification of the primary phloem and rooting capacity, the rooting of difficult subjects might be facilitated by using very young shoots taken before the cells of the primary phloem lose their living protoplasts. This method is, of course, exploited in propagation of softwood cuttings under mist where rapidly growing shoots are used. At this stage of development, wall thickening may have begun in primary phloem cells destined to become fibres, or sclereids, but lignification is usually absent, or slight, and the cells retain a living protoplast in all except the most shy-rooting kinds. In plants that are exceptionally difficult to root, even from softwood cuttings as, for example, beech, the onset of tissue senescence in the phloem appears to be very rapid, and, in addition to the formation of a sclerenchymatous cylinder in the primary phloem, the broad multiseriate secondary phloem rays may become lignified in quite young tissue close to the cambium (Fig. 3).

The simplest explanation of the effect of a sheath of lignified tissue on rooting capacity would be a mechanical one, but this is probably not the sole—or even the major—cause of failure to root since, in many plants, root initials do not form at all readily within such a sheath. Some alternative hypotheses are at present being investigated.

Quite apart, however, from speculations on the mechanisms involved, it would appear that the observed relationship between anatomy and ease of rooting may have a practical application and that, within certain limits, it may be possible to forecast rooting capacity from the structure of young stems.

Thanks are due to Dr. C. R. Metcalfe, keeper of the Jodrell Laboratory, Royal Botanic Gardens, Kew, for having suggested this investigation, and to Mr. R. J. Garner for providing much of the material and giving information on the rooting of a number of clones.

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LETTERS TO THE EDITORS

GEOPHYSICS

A Morning Discontinuity in the Orientation of Quiet Auroral Arcs

From all-sky camera recordings taken at this Observatory (geographic co-ordinates 67·8° N., 20·4° E.; geomagnetic co-ordinates 65·3° N., 115·5° E.) during the period December 1958 – March 1961 the directions of 366 quiet auroral arcs were measured. Only arcs extending from horizon to horizon, or at least covering an angle of view of about 100° for the most distant arcs and having an elevation angle of more than 15° for the highest point, were used in the analysis.

With the assumption of a constant height along the lower limit of the luminosity—for which there is good evidence (cf. Störmer[1])—the angle between a straight line connecting the points at which the arc intersects the horizon and a line through the midpoint of the chord, parallel to the meridian through Kiruna, was measured. Although this angle may differ slightly from that between the projection of the arc on Earth's surface and the local meridian through the midpoint of the arc—as measured by most investigators before the advent of all-sky cameras—the difference is estimated at less than one degree in the actual case. Since this difference is less than, or at the most equal to, the error introduced in measuring the angle of the chord, it has no appreciable influence on the results obtained. To the types of uncertainties mentioned should be added the possible error introduced in extrapolating some of the arcs to the horizon. Since this is estimated at 1–3°, the total error in the obtained direction of an arc will certainly not exceed 4°.

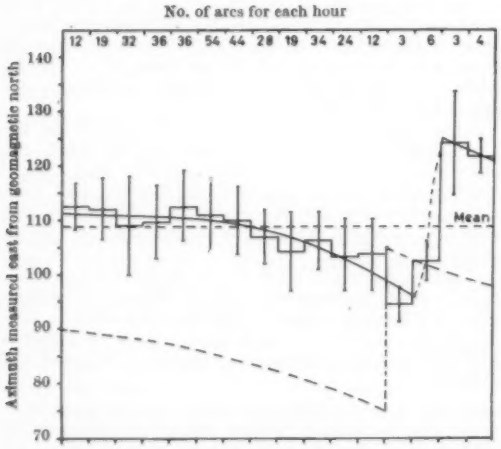

Fig. 1. Observed directions of quiet auroral arcs at Kiruna as a function of M.E.T. and of approximate geomagnetic time. The average azimuth and the standard deviation are given for each hour. The lower, dashed curve is Alfvén's theoretical diurnal variation curve

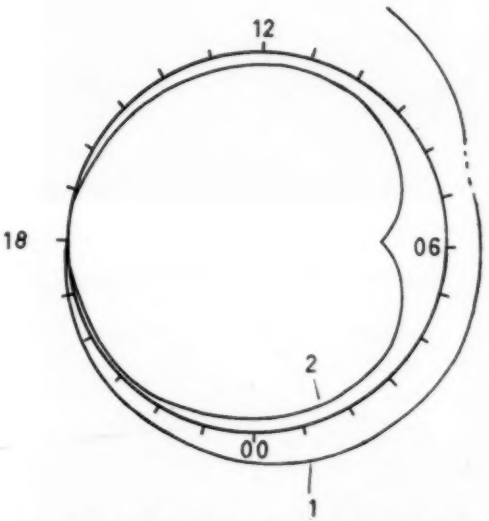

Fig. 2. Curve 1 is the auroral curve on Earth corresponding to Alfvén's I-curve, plotted from the diurnal variation in the orientation of quiet auroral arcs observed at Kiruna. It is corrected for the non-dipole part of the geomagnetic field. Curve 2 is Alfvén's theoretical I-curve

The result of the alignment investigation is shown in Fig. 1. For each hour of M.E.T. the average direction and the standard deviation were computed. In so doing, the direction value of each arc was weighted with the duration of the arc. The total average for the whole material was 109° and a typical standard deviation was 6°.

At the top of Fig. 1 the number is given of arcs used for each hour. The number has a maximum around geomagnetic midnight. In the morning hours the aurora as a rule is not suitable for direction determination, being usually diffuse and situated far to the north. It is not surprising, therefore, that the observational results are least for those hours. The morning arcs for which the direction was measurable were all of short duration.

Although the observational material is not extensive for the interval 0400–0800 M.E.T., it allows the conclusion that the very rapid increase of the azimuth occurring between 0500 and 0600 M.E.T. is significant.

The azimuth change in the morning is so rapid as to resemble a discontinuity of the type predicted by the Alfvén theory[2]. Such a marked 'discontinuity' has not, to our knowledge, been observed before. Lassen[3] observed a rapid change in direction at Godhavn at approximately the same local time, but the amplitude of the change was less (about 20° as compared with 30° in Kiruna).

In point of fact it should be possible to observe the discontinuity only in those longitudes in which geomagnetic time runs ahead of local time, since daylight will preclude auroral observation until 8 or 10 o'clock (geomagnetic time) in other longitudes.

From the smoothed diurnal variation curve of Fig. 1 the auroral occurrence curve—corrected for effects

of the non-dipole part of the geomagnetic field —corresponding to the 'ideal auroral curve' (the I-curve) of Alfvén[2] was plotted and is shown in Fig. 2 together with Alfvén's theoretical I-curve. The entire experimental curve is situated outside the circle, with which it coincides at 1800, in contrast to the theoretical curve, which is inside the circle. The difference is attributable to the fact that the azimuth values of the experimental curve are 7–9° greater than the values of the theoretical curve even after correction for the effects of the non-dipole components of the geomagnetic field. Hence, the theoretical curve is situated outside the standard deviation 'region' of the observational material. The difference, therefore, is probably a real one, which is not surprising since the theoretical curve is based on a highly idealized model.

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ASTROPHYSICS

Lunar Dust and the Gegenschein

OF the four possible explanations of the gegenschein which have been proposed, not one so far has explained satisfactorily the meagre observational data concerning this phenomenon. The Glyden–Moulton hypothesis[1], which suggests a concentration of meteoric material at a libration point of the Sun–Earth system, seems incapable of explaining all the luminosity of the gegenschein. The zodiacal light hypothesis[2], which claims that the glow opposite the Sun results from a phase-function for the interplanetary dust layer which produces such a brightening, fails to explain the fact that the gegenschein usually lies approximately 3° west of the anti-solar point[3]. The gaseous tail hypothesis[4,5], in explaining the light as the result of excitation of Earth-escaped gases by the interplanetary plasma, does not account for the fact that the colour of the gegenschein is slightly redder than that of the Sun[6]. Finally, the dust tail hypothesis[7], although explaining the facts in a qualitative way, at least, requires a large and continuous source of dust particles in Earth's neighbourhood.

Before the satellite measures of interplanetary dust concentration became available, there was little reason to suggest such a source of dust in the immediate vicinity of Earth. However, the remarkable fact that Earth is surrounded by a concentrated dust layer[8] seems to be explained in no other way. Whipple has found that the Moon might be the source of Earth's dust layer, for under certain conditions, the velocities of fragments thrown up from the lunar surface as a result of meteorite impact will exceed the lunar escape velocity. Many of these particles will be captured in quasi-stable orbits about Earth, forming just the sort of layer of dust found by the satellite experiments. Smaller particles will be blown out by radiation pressure before they can orbit Earth.

It seems reasonable to suggest that this localized source of dust in the immediate vicinity of Earth is sufficient to maintain a dust tail for Earth acted on probably by radiation pressure analogously with type II comet tails. If such is the case, then Earth has a dust tail as well as a gas tail[9], and we would be in the position of looking down the axis of the tail. To test such a hypothesis it will be necessary to work out theoretically the mechanism of supply of the dust layer and of its depletion. It will also be helpful to have further observations of the gegenschein to confirm its colour, spectrum, location, surface brightness, and variations in these quantities. Especially interesting would be systematic studies of the motions of the gegenschein and any relations these might have with other events. Measures of the parallax of the gegenschein should be attempted from an orbiting vehicle.

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PHYSICS

Mode Characteristics and Coherence in Optical Ruby Masers

AN optical maser, using a good-quality ruby crystal with plane-parallel reflectors, oscillates in modes corresponding to Fabry–Perot resonances. Oscillations are shown to occur at several different wavelengths corresponding to various numbers of half-wave-lengths between reflecting ends. For each wavelength, there are many angular modes which are coupled so that they oscillate coherently. This implies that a set of angular modes may be considered a single modified mode and that light from the entire set may be focused to a point of minimum size allowed by the wave-length of the light.

There have already been various discussions of the modes in an optical cavity[1–4] and experimental demonstration of a ring structure[5] in light emitted by ruby masers. The photograph shown in Fig. 1 was taken by passing a pulse from a ruby maser through a 410-mm. lens, with the photographic plate in the focal plane (far-field pattern). The series of rings observed corresponds within the accuracy of measurement with the pattern of a Fabry–Perot interferometer. Separations between successive sets of rings follow the interferometer equation:

$$D_n^2 - D_{n-1}^2 = \frac{4f^2\lambda\mu}{L}$$

where D_n is the diameter of the n^{th} ring on the film, f the focal length of the lens, λ the wave-length, μ the refractive index, and L the geometrical length of the ruby. The value of L obtained from this expression, on substitution of other quantities and measured values of D_n, agrees with the measured length of the crystal to within a few per cent. This was found to be the case for crystal samples of differing lengths, a fact which rules out interpretations of the rings on the basis of diffraction by some unidentified aperture.

The ring pattern of Fig. 1 was allowed to fall on two 100μ apertures separated by 2 mm. which accepted light from different rings or different portions of the same ring, corresponding to different angular directions of propagation within the ruby interferometer. Interference patterns characteristic of coherent sources resulted (Fig. 2), which reproduce the pattern found at a distance of 2·5 ft. with no lens interposed. This implies that various angular modes are of precisely the same frequency and coherent in phase. They must therefore be effectively coupled. Scattering from lattice imperfections is known to be prominent, and can be expected to couple the various modes as a result of transfer of light between them.

One must not, however, assume that only one frequency is present in the oscillation. The photograph in Fig. 3 was taken by interposing between the ruby and camera an external Fabry–Perot interferometer of about 1-cm. spacing. The ring structure of Fig. 1 is seen in the background of the various orders of the interferometer. The 'fine structure' components shown were measured and the wave-

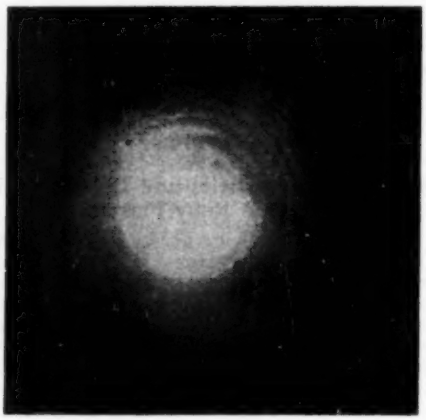

Fig. 1. Ring pattern formed by light beam from ruby maser at the focal plane of a lens. Spacing of rings corresponds to angular modes in ruby rod for a fixed frequency

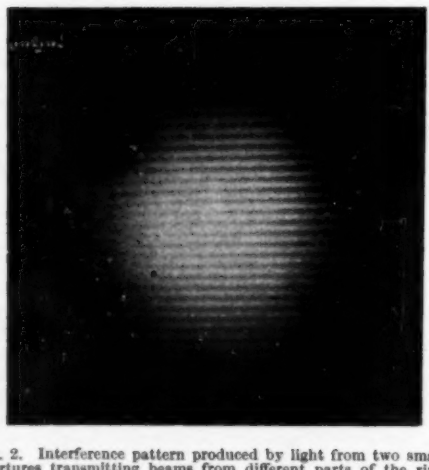

Fig. 2. Interference pattern produced by light from two small apertures transmitting beams from different parts of the ring pattern of Fig. 1

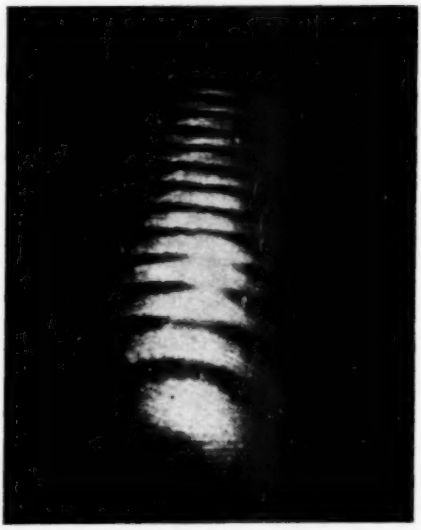

Fig. 3. Rings produced by beam from ruby maser after passage through a Fabry–Perot interferometer, showing resolution of longitudinal modes. The less obvious superimposed series of rings is the same shown in Fig. 1, which occurs without passage through a Fabry–Perot interferometer

number difference of adjacent components was about 0·07 cm.$^{-1}$ which, for our crystal, is equal within about 10 per cent to the separation of longitudinal modes $\Delta\nu = 1/2\mu L$. Such separations have previously been measured directly as beats in the helium–neon and ruby systems[6,7].

The several frequencies shown in the pattern of Fig. 3 are, of course, all within the ruby emission line, and are so close together that their interference patterns can coincide in Fig. 2. Hence the emission of the ruby maser is composed of several different frequencies with separations determined by those of the longitudinal modes. Each of these frequencies is emitted coherently in a series of coupled angular modes. The large number of angular modes spreads the emitted energy into a beam the width of which in solid angle exceeds that of the diffraction pattern of the principal mode by as much as several orders of magnitude[8]. We mean by a principal mode one in which the wave propagation is essentially perpendicular to the reflecting ends, and hence which can be expected to have the lowest losses[1–4]. It should be noted that oscillations under many different conditions always showed a maximum in the direction of the principal modes. Presumably, it is the lower losses of these modes, and their saturation of adjacent frequency regions in the ruby emission during oscillation, which produce just those frequencies that correspond closely to several adjacent principal modes in the cases examined. One must expect under some conditions to find other combinations of frequencies.

It is interesting to note that the coherence observed between the angular modes, and the coupling which must exist between them, implies that the set of angular modes may be regarded as a single modified mode, in which the maser is oscillating. This means, further, that it should be possible to concentrate the energy at a point or in a beam as small as that allowed by coherent oscillations in any other form of single mode. Such a possibility can be of importance

in concentrating large amounts of power from solid-state masers into small spots or highly directed beams. For example, one may note that the successive rings in the focal plane of the lens may be all made to arrive in phase at any arbitrary point on the axis by interposing a suitable phase-shifting plate at each ring. The light would then be focused as by a zone plate, and a suitable following lens could produce a beam of width limited primarily by the aperture of the lens.

The phase coherence of the various angular modes implies also the existence of rings in the near field due to interference of the beams from these modes, which may be considered as a set of coherent waves travelling at various angles with respect to the axis. Some rings have been observed in the near field by us and by others (Collins, R. J., private communication), but they have not been carefully compared with expected theoretical behaviour.

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de Haas–van Alphen Effect in InBi

The de Haas–van Alphen effect (oscillatory dependence of magnetic susceptibility on magnetic field strength at low temperatures) has provided one of the most successful methods for studying the Fermi surfaces of pure metals at low temperatures. Recently the utility of this technique has been extended to semiconducting compounds with the observation of the de Haas–van Alphen effect in degenerate *p*-type PbTe (ref. 1). In this communication we report what we believe to be the first observation of de Haas–van Alphen oscillations in a metallic compound, InBi (ref. 2).

The single crystal of InBi used in the present investigations was obtained by mixing stoichiometric quantities of indium and bismuth in a 'Pyrex' glass tube under vacuum. The molten compound was then forced under helium pressure into a tapered 'Pyrex' glass capillary which was allowed to cool slowly in a temperature gradient. A cylindrical sample of length 13 mm. and diameter 0·9 mm. was cut from the capillary with a sandblaster, and the glass casing was dissolved with hydrofluoric acid. An X-ray analysis showed the direction of the sample axis to be $33° \pm 2°$ from a $<100>$ axis in the basal plane of the tetragonal crystal. The pulsed field apparatus

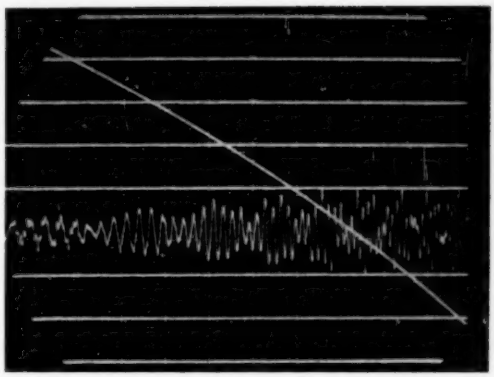

Fig. 1. de Haas–van Alphen effect in InBi at 1·11° K. The oscillating trace shows the output from a pick-up coil containing the sample. The curved trace shows the magnetic field increasing from 80·1 to 130·6 k.gauss during a sweep time of 1·8 msec. (time increasing from right to left). The beating effect is due to two oscillating terms of different period and amplitude

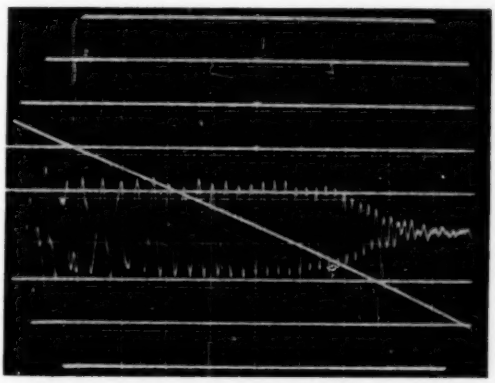

Fig. 2. Long period de Haas–van Alphen oscillations in InBi at 1·11° K. The diagonal trace shows the field increasing from 36·2 to 70·0 k.gauss during a time of sweep of 0·9 msec. The change in interval occupied by each successive oscillation is a consequence of the decrease of the time derivative of the magnetic field and the periodicity of the oscillations in H^{-1}

used in this work has been described briefly in previous communications[3,4].

With the sample mounted with its axis parallel to the magnetic field (within $\pm 2°$) we observed three oscillating susceptibility terms which are pictured in Figs. 1 and 2. The pertinent de Haas–van Alphen parameters for these three periods are given in Table 1. The extremal cross-sectional areas, A, of the Fermi surface were calculated from the periods, P, by the relation $A = 4\pi^2 e/chP$. The effective mass for each period was determined from the temperature-dependence of the amplitude of the oscillations[5].

It is apparent that the ranges of de Haas–van Alphen parameters in InBi are similar to those characteristics

Table 1. Periods, Corresponding Fermi Surface Extremal Cross-sectional Areas, and Effective Masses for de Haas–van Alphen Oscillations in InBi. (Accuracy of Periods is Approximately 5 per cent)

Period (10^{-8} gauss^{-1})	Area (10^{15} cm.$^{-2}$)	m^*/m_e
8·43	1·13	0·58 ± 0·1
6·33	1·51	0·64 ± 0·1
25·4	0·38	0·21 ± 0·05

of pure polyvalent metals[5]. The application to InBi of the nearly free electron model[6,7], which has met with some success in understanding pure metals, will be attempted when more data are available. de Haas–van Alphen measurements should be possible on a wide variety of metallic compounds, thus providing insight into the nature of electronic binding and structure in such materials.

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A Method of Calibrating Two-Sphere Non-directional Radiometers

When two heated spheres, one black and one bright, but otherwise identical, are kept at the same temperature, they lose an equal amount of heat by conduction and convection[1]. Any difference in heat input is due to the difference in radiational exchange. Neither air temperature nor air velocity enters the calculations for determining the mean radiant temperature of the surroundings. The quantities needed are: the heat input of the two spheres; the temperatures of the two spheres (which should be equal); the surface area; the emissivities of the bright and the black sphere. Nickel[1] or gold[2] is used for plating the bright sphere.

Attempts have been made to predict the accuracy of the two-sphere radiometer from the above quantities. Roose[1] reasoned that, as the heat input can be measured with an accuracy of ± 0·5 per cent, the error in mean radiant temperature would be ± 0·36° F. Sutton and McNall[2] anticipated errors not exceeding ± 2° F.

The method now proposed avoids the need to measure surface areas and emissivities. Advantage is taken of times and circumstances when the readings of a globe thermometer[3] and of air temperature happen to be equal. This is so, for example, out of doors in the evening during the transition from solar heat gain to radiational loss; or it may be so indoors under certain conditions. In such circumstances, it is clear that there is then, for the time being, a state of thermal equilibrium in which globe temperature and air temperature are both equal also to the mean ambient radiant temperature, so that the latter is known. Thus, if the two-sphere radiometer is operated at such a time, the answer which it should give is known, and only the heat inputs to the spheres need be measured, in addition, in order to enable its calibration constant to be found.

Using the above method it was found that the average error when measuring mean radiant temperatures with the Sutton–McNall[2] instrument was 0·4 deg. F.; the highest deviation encountered was 1 deg. F.

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BIOPHYSICS

Montmorillonite as a Caliper for the Size of Protein Molecules

It is known that proteins may be adsorbed from solution by the clay montmorillonite, leading to an increase in $d(001)$ crystallographic spacings[1]. In order to compare these increases with the size of protein molecules one must be certain that a complete mono-molecular layer of protein has been adsorbed, and this can be calculated to require 1·3–2·0 gm. of protein per gm. of clay. As the clay becomes saturated the adsorption isotherm levels off, but the isotherms are initially steeper than curves of the Langmuir type[2]. Assuming that protein molecules are spherical, we have calculated the expected $d(001)$ spacings and compared them with the experimental values found (Table 1). The agreement is moderately good for all but pepsin. Hæmoglobin appears to be adsorbed as a monomer, whereas lactoglobulin enters the clay particles as a dimer.

Table 1. Adsorption of Proteins by Montmorillonite

Protein	Mol. wt.	Adsorption (gm./gm.) Theoretical	Adsorption (gm./gm.) Experimental	Calculated mol. diam., Å	$d(001)$, Å. Theoretical	$d(001)$, Å. Experimental
Lysozyme (native)	14,700	1·3	1·3	32·6	42·2	44
(denatured)	14,700	—	1·3	—	—	46
Chymotrypsinogen	22,500	1·5	1·4	37·2	46·8	40
Lactoglobulin	35,000 (17,500)*	1·7	1·5	43·8 (34·8)	53·4 (44·4)	54 —
Pepsin	35,000	1·7	1·0	43·8	53	18
Hæmoglobin (human)	63,000 (40,000)*	2·0	1·9	53·2 (45·6)	62·8 (55·2)	53 —

* These proteins dissociate under extreme conditions of dilution and/or salt concentration to give the values in parentheses (ref. 3).

Our result with native pepsin agrees with that of Talibudeen[4], namely, that the $d(001)$ spacing is about 18 Å. for a wide range of amounts of protein adsorbed; it indicates an unfolding of pepsin molecules into extended polypeptide chains within the clay particles.

It has often been suggested that during heat denaturation of proteins there is a tendency for protein molecules to undergo unfolding or expansion. We have found that the $d(001)$ spacings of montmorillonite–lysozyme complexes are the same for adsorbed, heat-inactivated lysozyme as for native lysozyme, from which it may be concluded that lysozyme does not change drastically in shape on undergoing denaturation in solution.

Tobacco mosaic virus (isoelectric point 3·5) was allowed to react with sodium (Wyoming) bentonite at *p*H 3·2 (citrate buffer, ionic strength 0·0375). According to spectrophotometric analysis, 1,830 mgm. of virus was adsorbed per gm. of clay. At this value, about 15 per cent of the internal surface area

of the clay could be covered with the virus. However, X-ray data indicated that internal adsorption had not taken place.

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HYDROGRAPHY

Concentration of Chloride in Fresh Waters and Rain Water

A study of analyses of the waters of a number of freshwater lochs in the north-west of Scotland, sampled in 1955, showed that the chloride concentrations (Cl) varied with the distance (D) of the loch from the coast, the most suitable equation relating these parameters being of the form $Cl = K.D^{-n}$, where K and n are constants. This form of equation was used by Gorham[1] to describe similar data obtained from tarns in the English Lake District. A second series of lochs in north-western Scotland, sampled in 1960, and an earlier series of samples from lochs on the island of Rhum, western Scotland, taken in 1958, have also provided data fitting the general equation adequately.

For the Scottish lochs, which are situated near a very irregular coastline, the parameter D is estimated in a south-westerly direction (that of prevailing strong winds) to the coast exposed to open sea. Despite the necessarily imprecise measurement, it has been found that the coefficients of correlation r for the regression of chloride concentration on D, after logarithmic transformation, are close to unity. Furthermore the various values obtained for the constant n are in close agreement. The values of the proportionality constant K differ to some extent, but are nevertheless of a similar order. The results are summarized in Table 1, in which other relevant information is included.

The samples of loch water in each series were all taken within short periods of one month or less, in areas where chloride derived from terrestrial sources is insignificant. All chloride is consequently derived from cyclic salts dissolved in rain water, or directly from sea-spray droplets. The analyses of loch water can thus be considered as the integrated values of total precipitation (including sea-spray) draining from the catchment areas of the lochs, the period of accumulation being virtually the same for all lochs in any one group.

In work of mine on the rainfall of the island of Rhum it has been shown that the sodium content of the total precipitation at one sampling station (Kinloch) was linearly related to the maximum gust velocity of the wind in the area during the appropriate sampling period, for velocities above a threshold of about 30 knots. The chloride/sodium ratio varied little from that of sea-water. It seems possible that the proportionality constant K depends at least partly on the wind velocity, the prevalence of high wind velocities in a particular area over a definite period of time giving high values of K.

In addition to the four series of data from freshwater lochs, constants have also been calculated from two series of rainfall analyses taken from the references quoted and are included in Table 1. The values of n for these two series are similar to those calculated for the lochs. They are derived from mean values of chloride concentrations in precipitation sampled regularly over long periods, and such mean values can be considered as analogous to the loch water analyses.

The mean value of the constant n, for the six series of data in Table 1, weighted according to the number of samples, is 0·328, and the equation thus approximates closely to the form $Cl = K/\sqrt[3]{D}$. The correlation coefficient is high in all six series ($P < 0{\cdot}001$), and the relationship appears to be valid for distances ranging from 0·1 to 80 km., and probably much longer. It is suggested that the marine influence on fresh water is governed by such a relationship.

Other attempts have been made to derive more complex relationships for data of the type discussed, variants of the general equation $Cl = ae^{-pD} + be^{-qD}$ being used (where a, b, p and q are constants). Eriksson[2] applied this equation to the data of Leeflang[3] while Conway[4] used a similar type of equation, with the addition of a further constant, to Jackson's[5] data. The four constants in Eriksson's formula differ from the corresponding constants in Conway's formula. An attempt to fit the Scottish data to an equation of the Eriksson type yielded constants differing from those of both Eriksson and Conway. It is considered that the simpler formula $Cl = K.D^{-n}$ has the merit of consistency in that similar values of n have been deduced from data obtained in widely differing regions, while the values of K are of a similar order, and are possibly related to the mean wind velocity in the area and sampling period concerned.

No theoretical derivation of the proposed formula has been attempted, but it is probable that deviations can be expected at very short or very long distances. The deduced value of chloride concentration at zero distance would be infinitely large, perhaps represented by salt particles, although in practice this would not be found. Topographical features no doubt also have some influence on the salt depositions. While the proposed formula is doubtless a simplification of more complex equations which may govern the pheno-

Table 1

Location	No. of sampling stations	Range of chloride concentration (Cl) m.equiv./l.	Range of distance (D) km.	Sampling period	r	K	n	Reference
North-west Scotland	21	0·51–1·59	0·8–31	6 days, 1955	−0·887	1·49	0·336	Holden
North-west Scotland	20	0·39–1·07	1·3–14·5	7 days, 1960	−0·890	1·21	0·334	Holden
Rhum	10	0·38–1·39	0·2– 5·8	9 days, 1958	−0·989	0·74	0·346	Holden
Lake District	16	0·19–1·80	0·1–51	1 month, 1957	−0·912	0·67	0·303	Gorham (ref. 1)
New Zealand	11	0·09–0·63	0·5–61	2 years rainfall	−0·914	0·46	0·341	Gibbs and Blakemore (ref. 6)
Holland	6	0·09–0·46	0·44–86	6 years rainfall	−0·967	0·32	0·287	Leeflang (ref. 3)

menon of salt transport, it does suggest that such transport from the oceans is a continuous process extending over considerable distances, rather than the combination of direct transport of spray droplets over short distances near the coast, and the solution of cyclic salts by precipitations alone at all other distances as could be implied from equations of the Eriksson or Conway type.

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METALLURGY

A New Reversible Solid-State Transformation in Iron–Nickel Alloys in the Invar Range of Compositions

UNUSUAL physical properties exhibited by alloys of the iron–nickel system in various ranges of composition have led to extensive investigations[1] of this system in the past. One group of alloys is that contained in the invar range, namely, 30–45 atom per cent nickel, and is characterized by unusual thermal expansion, magnetic and other physical properties. In spite of extensive investigations, the general sluggishness of solid-state transformations in the iron–nickel system has prevented true equilibria from being well established. The properties of these alloys, therefore, have remained largely unrelated to their structural and/or crystallographic features.

During a systematic re-examination of solid-state equilibria in the iron–nickel system, using new techniques capable of yielding true equilibria within reasonable lengths of time, we have found a new reversible transformation which occurs in these alloys in the temperature-range of ~ 150°–300° C. In the present communication, this transformation is described and its possible bearing on the physical properties of the alloys discussed.

Equilibrated iron–nickel alloys containing up to 75 atom per cent nickel were prepared in the form of pre-alloyed ultra-fine powders by the thermal decomposition and hydrogen-reduction of iron–nickel mixed-crystal formates[2,3] at various temperatures, including temperatures as low as 200° C. Most of these alloys, as-quenched to room temperature from their respective temperatures of preparation, generally showed the equilibrium structures corresponding to the temperature of preparation. However, alloys with 30–45 atom per cent nickel showed an exceptionally different behaviour which, on detailed investigation, proved to be due to the occurrence of the new transformation. On re-heating, all the as-quenched alloys readily became transformed to yield equilibrium structures corresponding to the temperature of holding.

As-quenched alloys were examined by X-ray diffraction at room temperature, using a Philips diffraction camera of 114·7 mm. diameter. High-temperature X-ray diffraction patterns were next obtained with a Unicam high-temperature diffraction camera which had been previously calibrated against the thermal expansion of gold. Using a high-intensity rotating-anode X-ray generator, diffraction patterns could be secured with exposures as short as half an hour. Cobalt and iron characteristic radiations were generally used, manganese radiation being used in those cases where high resolution was sought.

Fig. 1. Room-temperature X-ray diffraction pattern showing (311) back reflexion from a 31 atom per cent nickel iron–nickel alloy; alloy quenched to room temperature from the γ-state (~ 600° C.). Manganese $K\alpha$ radiation

Examination of the diffraction patterns revealed that the transformation occurs in all the face-centred cubic γ-phase alloys which contain approximately 30–45 atom per cent nickel. When alloys of this structure and composition are quenched to room-temperature from the γ-phase, their X-ray diffraction lines always appear broad (Fig. 1). That this broadening is not to be associated with quenching strains or with effects of fine particle-size became evident from high-temperature X-ray diffraction investigations during thermal cycling of the as-quenched alloys. On heating to or above what appeared to be a critical temperature, the broad diffraction lines of the quenched alloys showed a pronounced sharpening. Subsequent cooling resulted in loss of sharpness of the lines and the reappearance of their broadening (Fig. 2). The effect has been reproduced repeatedly and takes place readily.

Closer examination of the broad lines indicated that the broadening may be interpreted as due to the superposition of two closely spaced reflexions having the same indices but arising from two face-centred cubic phases (designated as γ_1 and γ_2) of slightly different lattice constants. The sharpening of the lines on heating may thus be attributed to the coalescence of the two γ-phases into a single γ-phase.

In spite of the poor resolution of the X-ray diffraction lines, an attempt was made to measure the lattice parameters of the two γ-phases. Typical results are shown in Table 1, from which it may be seen that the difference in the lattice constants is only about 0·008–0·009 Å.

Transformation temperature appears to be dependent on the composition of the alloy. Exact transformation temperatures for various compositions could not be determined, but they seem to lie in the

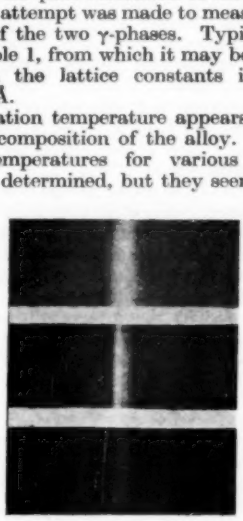

Fig. 2. High-temperature X-ray diffraction patterns showing (311) back-reflexion from a 31 atom per cent nickel iron–nickel alloy; alloy as-quenched to room temperature from the γ-state subjected to thermal cycling. Iron $K\alpha$ radiation

Table 1. Lattice Constants of the Two γ-Phases co-existing at Room Temperature in Iron–Nickel Alloys with 31 and 36 Atom per cent Nickel

Atom per cent nickel in alloy	Lattice constants (Å.) γ_1	γ_2
31	3·593	3·585
36	3·593	3·584

approximate range 150–300° C. Note that the transformation occurs at relatively low temperatures. Considering the general sluggishness of solid-state transformations in most iron–nickel alloys, rendered all the more sluggish at temperatures below 500° C., the reversibility of this transformation is indeed very striking.

Observed features of the transformation are consistent with the interpretation that the transformation is of the magnetic kind, more specifically, ferromagnetic ⇄ anti-ferromagnetic. Such a transformation may serve as the key to the proper understanding of the anomalies in thermal expansion, magnetic and other physical properties, for which the 30–45 atom per cent nickel iron–nickel alloys are noted. The broad X-ray diffraction lines observed may thus be interpreted as due to the co-existence of ferromagnetic and antiferromagnetic modifications of the γ-phase in these alloys at these low temperatures. Taking into account exchange interactions between iron–iron, nickel–nickel and iron–nickel atom pairs in iron–nickel alloys, the possible occurrence of such a transformation in precisely the same composition-range as of the alloys of this work has recently been considered by Kondorskii[4].

The validity of our interpretation of data, however, needs further confirmation through more refined experiments using methods other than X-ray diffraction, for example, magnetic analyses as well as neutron diffraction. Work on these lines is now in progress.

In attempting to correlate the changes of properties, for example, thermal expansion, saturation magnetization, etc., of these alloys with composition and structure, we are led to pose the question: Should not magnetic transformations also be viewed as regular phase changes and therefore be given a fitting place in phase diagrams? This question has far-reaching implications. For example, if magnetic transformations are considered as regular phase changes, such concepts as 'solid phase' and 'crystal symmetry' will have to be modified to recognize the magnetic state of matter and the atomic symmetries associated with this state.

Results discussed here together with unreported results covering compositions up to 75 atom per cent nickel, indicate that the iron–nickel equilibrium diagram is much more complex than the most recent diagram[5] drawn for this system. We have constructed a new iron–nickel phase diagram which will be reported in the near future.

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CHEMISTRY

Relaxation Enhancement by Paramagnetic Ion Binding in Deoxyribonucleic Acid Solutions

The effect of paramagnetic ions on proton relaxation times depends not only on their concentration but also on the binding of the ion to other molecules which may be present in the same solution. By measuring longitudinal relaxation times (T_1) by nuclear magnetic resonance we have compared the proton relaxation-rates at constant ion concentration with and without deoxyribonucleic acid (DNA) present. In this way it was possible to detect binding and to distinguish between interior and exterior DNA binding sites for different transition metal ions.

In the present experiment T_1 was measured by the Carr–Purcell method[1] with a conventional spin-echo apparatus operating at 50 Mc./sec., for a wide range of magnetic ion concentrations in water and in calf-thymus DNA solutions.

The Solomon[2]–Bloembergen[3] expression for T_1 in the presence of paramagnetic ions is:

$$\frac{1}{NT_1} = \frac{4}{30}\frac{S(S+1)g^2\beta^2\gamma^2_I p}{r^6}\left[3\tau_c + \frac{7\tau_c}{1+\omega_s^2\tau_c^2}\right] + \frac{2}{3}\frac{S(S+1)A^2 p}{\hbar^2}\left[\frac{\tau_e}{1+\omega_s^2\tau_e^2}\right]$$

in which N is the molar concentration of paramagnetic ions; γ_I the proton gyromagnetic ratio; p the probability that a proton is in the hydration sphere of the paramagnetic ion in a $1M$ solution of the ions; τ_c the correlation time for dipolar interaction; ω_s the angular electron precessional frequency; A the isotropic indirect hyperfine interaction; τ_e the correlation time for the isotropic hyperfine interaction (electron spin relaxation time, τ_s, or correlation time for proton chemical exchange in the hydration sphere, τ_h, whichever is shorter) and r is the ion-proton internuclear distance.

At high fields, such as were used in the present experiments, the second term is negligible. The correlation time τ_c is given by $1/\tau_c = 1/\tau_r + 1/\tau_h + 1/\tau_s$, where τ_r is the rotation correlation time.

Consider how $1/NT_1$ is affected by paramagnetic ions originally in solution being bound to a molecule. If the ion is bound to a single point (that is, not chelated) on the outside of the molecule, two degrees of rotational freedom are eliminated and the third may be reduced by the 'friction' of the hydration sphere against the molecule. If the ion is chelated, rotation will in general be stopped completely. In any event $\tau_r^*/\tau_r > 1$, where the asterisk denotes values for the bound ions. If τ_s and τ_h are greater than τ_r and do not change appreciably when the ion is bound, as is often the case, then $\tau_c^*/\tau_c > 1$. The number of water molecules in the hydration sphere of the bound ion is reduced slightly so that $p^*/p \approx 5/6$. In this case of large values of τ_s, therefore, the rate of proton relaxation in the presence of externally bound ions is greater than when the same concentration of ions is in solution. If we define a relaxation enhancement factor $\varepsilon = T_1/T_1^*$ then $\varepsilon > 1$. This case is illustrated in Fig. 1 for aqueous solutions of Mn^{++} ions and DNA.

The second case is $\varepsilon < 1$, implying that p/r^6 and/or τ_c are reduced when the ion is bound; this has been used in several recent experiments[4]. The first alternative clearly indicates that the ion site is an interior one and not accessible to hydration water. The latter can only arise from a large decrease in

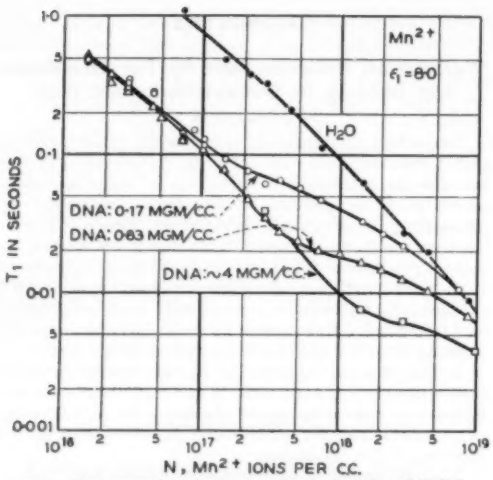

Fig. 1. The curves show the proton T_1 as a function of Mn^{2+} ion concentration in pure water and DNA solutions of various concentrations. The relaxation enhancement factor ε is approximately 8·0 for Mn^{2+} concentrations below the saturation value of 7×10^{17} Mn^{2+}/mgm. DNA, which agrees with conductiometric measurements (ref. 5)

electronic relaxation time on binding so that τ_s^* dominates τ_c^* (that is, $\tau_s^* < \tau_c$). In many cases even a rough knowledge of τ_s and τ_s^* makes the distinction between exterior and interior sites unambiguous.

Our experimental results for aqueous DNA solutions may be summarized as follows:

(1) For Cu^{2+}, Mn^{2+} and Cr^{3+} the measured values of ε are 10, 8 and 4·7, respectively. We conclude that these three ions are bound to exterior DNA sites, probably to the phosphate groups.

(2) For Fe^{3+} ε is 0·3 and if, as can be shown to be the case for the previously mentioned ions, $\tau_s \approx \tau_s^*$, we can conclude that Fe^{3+} is bound to an interior site.

(3) Ni^{2+}, Fe^{2+} and Co^{2+} show $\varepsilon = 1$. Since these ions are known to have a very short τ_s, we can only conclude that they are either not bound to DNA or bound to an exterior site with $\tau_s^* = \tau_s$. Recent experiments indicate that for Ni^{2+} and Co^{2+} the latter is the case[6,7]. The number of available metal ion sites was found to be approximately 7×10^{17}/mgm. of DNA for Mn^{2+}, Cr^{3+} and Fe^{2+} ($\sim$ 0·6 phosphate equivalent).

A detailed account of these experiments, which includes a discussion of the effect of the ionicity of the solution on the metal-DNA system, is being prepared.

The experimental method described here should be generally applicable to other problems of paramagnetic ion bonding to large molecules. An expression for $1/NT_2$ analogous to $1/NT_1$ leads to T_2 enhancement, which proves useful in the investigation of some ions.

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Detection by Ionization of Gases in Helium used in Gas Chromatography

The recent communication of Karmen *et al.*[1] on analysis by gas chromatography illustrates an interesting mode of operating a Lovelock ionization detector. The results given, however, do not indicate that the behaviour of the cell is in any way different from that used in the conventional manner. The cell used by me[2] has a similar electrode configuration and the results, where comparable with Karmen's, are practically identical (see Table 1).

Table 1

	Concentration (volumes per million) for maximum conductivity		Relative responses for equal volumes		
	Karmen	Wiseman	Karmen	Wiseman (ref. 2)	(a)
Hydrogen	—	~1,000	—	1	1
Nitrogen	520	750	4	3	~5
Argon	960	> 170	3	5	5
Oxygen	280	250	11	10	10
Methane	—	⩾ 100	—	20	~10
Krypton	—	—	—	—	~18
Xenon	—	—	—	—	20
Carbon dioxide	80	170	5	70	—

(a) Previously unpublished results (Wiseman) using microargon detector.

Some allowance must be made for the greater impurity of their helium, particularly in the case of carbon dioxide. It is therefore not possible to accept their contention that the design of cell is more important than the purity of the helium.

There are several points of considerable importance raised by their communication. First, their implication that a cell specially designed for use with helium is likely to work better than one based on work with argon is almost certainly correct. Secondly, to obtain the best results, a leak-tight detector is essential, but the short outlet illustrated in their diagram would appear to be inadequate to prevent back-diffusion once helium of really high purity was used. Thirdly, there are some serious implications overlooked in their acceptance of helium of commercial grade without further purification. It is well known that bottles of commercial gases vary substantially in trace composition and in particular in water content. Water appears to have an even higher response in these detectors than carbon dioxide. Further, it is clear from Table 1 that were carbon dioxide the major constituent of the 100 v.p.m. impurity-level of the commercial helium, the helium would not give increases of conductivity with any gases. Hence a mere specification of 99·99 per cent would be meaningless without some data on the nature of the impurities. It is apparent from published data[1,2] that the tolerance of gases like argon and hydrogen is about 10 times that of carbon dioxide and probably water. Karmen *et al.* do not state whether they dry their helium. If they did, then possibly their specification is much better than indicated. Even one molecular sieve trap in liquid nitrogen is a very effective purifier.

Table 2

	Tolerable impurity-level (v.p.m.)
Neon	10 (?)
Argon	10
Hydrogen	10
Nitrogen	5
Oxygen	3
Carbon dioxide	1
Water	< 1
Hydrocarbons	1
Carbon monoxide	1
Total impurities	⩾ 20

These figures are based on 1 per cent of the figure for maximum conductivity.

Further disadvantages of using helium of relatively low purity are the reduction of the linear range—possibly even its complete elimination and the irregular calibration curve likely to result. This could be S-shaped in character, and in any event as the conductivity/concentration curve reaches a maximum, the sensitivity of the device to changes of concentration reaches a minimum.

In general, therefore, users should be recommended to obtain the purest helium possible compatible with reasonable trouble in purification. A suggested specification for commercially available pure helium is given in Table 2.

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Quantitative Estimation of Peak Areas in Gas-Liquid Chromatography

RECENTLY, Carroll[1] advocated the use of the product of retention time (t_R) and peak height (h) for the quantitative estimation of relative peak areas. This method[2], based on the assumption of a proportionality of peak width (w) and retention time (t_R) or volume (V_R), gives satisfactory results with respect to the reproducibility. In fact, we have found that the reproducibility (under equal operating conditions) of this method is better than that of any other 'manual' estimation of peak areas. However, the method is not commonly used in our Laboratory, as the results obtained with it are erratic. This is due to the fact that in most cases the proportionality between w and V_R is only approximate. Differences between the relative areas, as found by the triangulation method and from the product of h and V_R, can amount to several per cent relative. In principle the non-linearity of w and V_R can be taken into account when calibrating the detector. However, changing the column or altering the carrier gas velocity will change the calibration factors.

Equation (17) in ref. 3 can be written as:

$$C_v = \frac{n^{1/2}}{V_R\sqrt{2\pi}} \exp\left(-n(V-V_R)^2/2V_R^2\right).\, a \qquad (1)$$

where n is the number of theoretical plates, V_R is the retention volume, V is the volume of carrier gas passed through the column since the introduction of the sample, C_v is the concentration of the component in the carrier gas, a is the amount of the component in the sample.

From this equation it follows that (for a Gaussian peak):

$$h \times V_R \simeq \frac{n^{1/2}}{\sqrt{2\pi}}\, a \qquad (2)$$

Since n depends *inter alia* on the distribution coefficient[4], and thus on the retention time of the component, the relation between the product of h and V_R and the amount a will, in general, not be the same for every component. Therefore the method can only be expected to yield good results when the differences in retention volumes are very small, or when the C-term in the van Deemter equation is relatively unimportant, that is, when the linear gas velocity and the amount of stationary phase are small (influence of diffusion coefficients on n being neglected). In Carroll's measurements this is certainly not the case as the relative areas of the early peaks are larger when the triangulation method is used, whereas the relative areas of the late peaks are larger (with one exception) when the estimation is made from the product $h \times t_R$. Most likely in these experiments the distribution coefficient of the most volatile component (methyl palmitate) was larger than the ratio of the volumes of mobile and stationary phases in the column. In consequence the number of theoretical plates must be expected to increase with the retention time. This will explain the discrepancies between the results obtained by the different methods. The same trend can be observed from the results summarized in Table 1.

Table 1. AVERAGE RELATIVE AREAS OF SEVEN PEAKS IN A CHROMATOGRAM OF A HYDROCARBON MIXTURE

Distribution coefficient of component	Column length / Sample volume / Method	2 m. 2·5 μl. $h.w$*	2 m. 2·5 μl. $h.V_R$	2 m. 5 μl. $h.w$*	2 m. 5 μl. $h.V_R$	4 m. 2·5 μl. $h.w$*	4 m. 2·5 μl. $h.V_R$	4 m. 5 μl. $h.w$*	4 m. 5 μl. $h.V_R$
55		2·7	2·6	2·7	2·6	2·8	2·5	2·8	2·5
72		13·8	13·8	13·6	13·7	13·7	13·5	13·7	13·6
148		12·6	11·8	12·6	11·7	12·3	11·4	12·5	11·4
172		7·6	7·2	7·7	7·3	7·2	7·1	7·3	7·2
206		46·0	46·7	45·8	46·6	46·2	47·2	46·3	47·1
267		6·3	6·3	6·4	6·4	6·4	6·3	6·2	6·3
380		11·1	11·7	11·2	11·8	11·5	12·0	11·3	11·9

* w, width at half-height.

Column, squalane on sterchamol (20: 100); temperature, 50° C.; ratio mobile to stationary phase, 6 (approximately).

The Perkin–Elmer *ABC* test mixture (hydrocarbons) was run on a 2-m. and on a 4-m. squalane column using 2·5 and 5 μl. samples. The relative areas of seven peaks of this mixture, given in Table 1, are the averages of the values obtained from five chromatograms.

It appears that for accurate quantitative analysis the method advocated by Carroll is not suitable.

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IN proposing the method referred to by Dijkstra, I tried to stress its convenience rather than its extreme accuracy and the examples which I gave were chosen to show that some lack of agreement might be expected in comparing areas determined by this method with areas determined by other methods. However, as Dijkstra points out, results obtained with other methods are less reproducible. Slight differences in the way in which triangles are drawn and small inaccuracies in cutting out curves can give rise to appreciable differences in estimated area. It is also more difficult to measure peak widths at half height accurately than it is to measure retention times. Furthermore, in the case of overlapping peaks it is frequently impossible to get a good estimate of the individual areas by other methods whereas the retention times and peak heights can still be measured and appropriate corrections for peak height applied if necessary.

A convenient and rapid method with somewhat limited accuracy can still be useful in work where

extreme accuracy is not required or where accuracy cannot be obtained in any event because of errors introduced by other steps in the analytical procedure. However, it is always desirable to improve the accuracy of a convenient method, and this can sometimes be done when sources of inaccuracy are known. For this reason I welcome Dijkstra's critical comment on the method for estimation of peak areas.

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Behaviour of 5A Molecular Sieve in Subtractive Gas Chromatography

5A molecular sieve can be used to remove selectively normal paraffins from hydrocarbon samples in the gas phase at the inlet of a gas liquid chromatographic column[1,2]. This subtraction technique has formed the basis of analytical procedures for the determination of the normal paraffins in complex petroleum fractions and has been applied in conjunction with both packed and capillary columns[3].

It has also been shown that at high temperatures (c. 300° C.) normal paraffins could be removed in a similar manner from waxes but in this molecular weight-range only 2-methyl eicosane and the highly branched paraffin squalane were available to test the selectivity of the molecular sieve. Neither of these compounds was sorbed by the sieve at a temperature of 320° C.

Recently, Murray *et al.*[4] have reported that unspecified single methyl branched paraffins in the wax range are completely removed from the gas phase by 5A sieve. This affinity for the singly branched paraffins was destroyed by repeated passage of these compounds through the sieve until no further sorption occurred. However, the sieve was still capable of retaining the normal paraffins.

We have now studied the behaviour of the single methyl branched hydrocarbons in greater detail using in particular a sample of 2-methyl dotriacontane synthesized in our laboratories[5]. In these experiments a pre-column 6 cm. long and 6 mm. internal diameter was packed with activated 30–60 B.S. mesh 5A molecular sieve. This pre-column was attached to the inlet of an 86-cm. gas-liquid chromatography column, also of 6 mm. diameter, containing 26 per cent wt. of silicone grease *E*.301 on 52–60 B.S. mesh 'Sil-O-Cel' firebrick. The pre-column was maintained at 320° C. and the gas-liquid chromatography column at 296° C. These conditions had been used previously for wax separations and were known to be satisfactory for such mixtures[3]. 10-μl samples of pure paraffin hydrocarbons were introduced into the pre-column and their behaviour is shown in Table 1.

At C_{33} the 2-methyl paraffin is completely sorbed whereas at C_{34} and below this type of compound is not sorbed by the sieve. Also, when the branching occurs in the middle of the molecule the C_{44}-branched paraffin is retained, but the similar C_{26} compound is not sorbed. At some critical chain-length between the limits indicated, single methyl branched paraffins start to be sorbed by the 5A sieve.

Table 1. Behaviour of Paraffin Hydrocarbons in a Gas-Liquid Chromatography Pre-column containing 5A Molecular Sieve

Completely sorbed from the gas phase		Not sorbed from the gas phase	
Docosane	C_{22}	2-Methyl eicosane	C_{21}
Tetracosane	C_{24}	2-Methyl tricosane	C_{24}
Hexacosane	C_{26}	13-Methyl pentacosane	C_{26}
Octacosane	C_{28}		
Dotriacontane	C_{32}		
2-Methyl dotriacontane	C_{33}		
22-Methyl tritetracontane*	C_{44}		

* Gas-liquid chromatography column temperature, 350° C.

Further work on the selectivity of the sieve will be carried out as more reference hydrocarbons in this range become available.

We wish to thank Mrs. M. Colwell for the synthesis of some of the paraffins used in the work.

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Improved Gas Chromatography of Unesterified Fatty Acids

It has been demonstrated by Böttcher *et al.*[1] and by Hunter *et al.*[2] that unesterified fatty acids can be separated, by gas chromatography, by the use of 'liquid' phases of the polyester type. Metcalfe[3] has pointed out the necessity to have a highly acidic 'liquid' phase in order to suppress the association of the carboxyl group of the fatty acids, and has shown that the separation of C_4–C_{22} fatty acids is very much improved by the incorporation of phosphoric acid in the polyester 'liquid' phase. Using a column packing of 'Celite 545' previously coated with 25 per cent of diethylene glycol adipate polyester and 2 per cent of phosphoric acid, mixtures of fatty acids were separated at temperatures of 220–235° C., yielding well-defined symmetrical peaks and separating saturated and unsaturated acids from each other.

The development of the argon ionization detector has resulted in higher sensitivities of detection, allowing a reduction of sample size. This reduction of sample size itself improves column efficiency and, in addition, allows the use of columns with lower content of 'liquid' phase, which in turn leads to improved resolution and a faster analysis. A faster analysis is particularly important with the separation of fatty acids which are more thermo-labile than their corresponding methyl esters. However, the choice of solid support is important when low ratios of 'liquid' phase to solid support are considered. The use of a solid support having a large surface area per unit weight, such as 'Celite', is not particularly suitable in some respects, since treatment with the 'liquid' phase could leave much of the surface area uncoated, allowing the solid support to act as an absorbent and thus to interfere with the distribution of the analysis sample between the 'carrier' gas and the liquid phase.

Bohemen and Purnell[4] have discussed the requirements of an efficient solid support. These authors have shown that, as predicted by the van Deemter, Zuiderweg and Klinkenberg rate equation[5], high column efficiencies are attained when the columns have the following characteristics: (*a*) narrow range of particle sizes; (*b*) spherical shape of particle; (*c*) small quantity of 'liquid' phase.

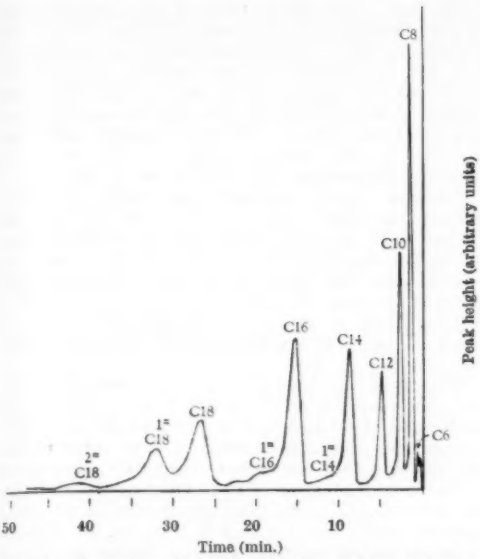

Fig. 1. Chromatographic separation of a mixture of C_6—C_{18} fatty acids. Sample size, 0·1 μl. of a 50 per cent solution in cyclohexane. Column temperature was 180° C., column size 1·2 m. × 4 mm.; argon pressure was 12 p.s.i. at column inlet, atmospheric pressure at outlet

All these requirements can be fulfilled by using small glass beads of uniform size, which can retain up to 3 per cent of 'liquid' phase by surface tension.

We have demonstrated that mixtures of unesterified C_6–C_{18} fatty acids can be analysed at temperatures ranging from 140° C. to 200° C., by the use of a glass-bead support coated with a mixture of 1 per cent of poly-ethylene glycol succinate and 0·4 per cent of phosphoric acid.

Poly-ethylene glycol succinate was prepared by heating a mixture of 1·0 mole of ethylene glycol and 1·05 moles of succinic acid at 150° C. for 3 hr. The resultant viscous liquid solidifies at room temperature and has a melting point of 80–82° C. The polyester was used as 'liquid' phase without further treatment. The polyester (0·5 gm.) was dissolved in chloroform (30 ml.) and to the solution was added, with constant stirring, 50 gm. of glass beads (Ballotini, No. 13 diam. = 0·075 − 0·1 mm., manufactured by the English Glass Co., Ltd., Leicester), followed by 0·2 gm. of phosphoric acid (85 per cent H_3PO_4). The stirring was continued while the chloroform was allowed to evaporate at room temperature. When the packing was almost dry, it was finally rendered solvent-free by heating for 0·5 hr. in an oven maintained at 100° C. The coated beads were then sieved, and only the portion passing a 85–100 mesh sieve was used to pack the column. The 1·2 m. × 4 mm. glass column was vibration-packed with the coated beads in the normal way. The packed column was purged for 72 hr. with a stream of argon, while the column was maintained at a temperature of 200° C. For fatty acid analysis, the Pye gas chromatography apparatus was used, which utilizes argon as carrier gas and strontium-90 as the radioactive source for the ionization detector. The mixtures of fatty acids analysed were dissolved in cyclohexane (50 : 50 w/v) and 0·1 μl. of the solution was used for each experiment. Normally, the detector voltage was 1,000 and the sensitivity control was set at × 10.

Table 1. Retention Times (min.) of C_6—C_{18} Saturated Fatty Acids at Various Column Temperatures

Saturated fatty acids	Column temperature (° C.)						
	140	150	160	170	180	190	200
C6	3·0	1·6	1·2	0·6	—	—	—
C8	6·4	4·0	2·8	1·6	1·4	1·0	—
C10	13·2	8·0	5·4	3·4	2·8	1·8	1·0
C12	27·0	16·0	10·6	6·6	5·0	3·0	2·0
C14	54·0	31·8	20·2	12·4	9·0	5·6	3·8
C16	107·0	62·0	37·2	22·4	15·2	9·8	6·2
C18	214·0	122·0	69·2	40·4	26·2	16·2	10·4

The separation of a mixture of C_6–C_{18} fatty acids, at 180° C., is shown in Fig. 1. The approximate retention times of each of the component acids of this mixture, at various column temperatures, are given in Table 1. This illustrates that long-chain fatty acids can be separated rapidly at comparatively low temperatures.

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BIOCHEMISTRY

Oxidized Cytochrome and Chlorophyll C_1^+ in Photosynthesis

Chance *et al.*[1a] reported the presence of trapped oxidized cytochrome in bacteria at −200° C., and in a previous communication[1b] we supplied evidence for the presence of trapped oxidized cytochrome during photosynthesis of green plants at −150° C. We have now refined and extended the measurements over the whole range of the visible spectrum. The difference spectrum of the trapped products in fresh spinach chloroplasts is shown in Fig. 1*a*. It shows in addition to the oxidized cytochrome Cyt^+ (solid line) the trapping of what is probably oxidized chlorophyll C^+ (dotted line). Both substances are products of one and the same light reaction $h\nu_1$. It has been shown that in green plants photosynthesis is triggered by two light reactions $h\nu_1$ and $h\nu_2$, one being sensitized by a chlorophyll pigment already sensitive at 710 mμ and the other sensitized by a chlorophyll pigment (P) sensitive at shorter wave-lengths (see refs. 2 and 3). Exciting only $h\nu_1$ (at +20° C. and 710 mμ) gives almost the same difference spectrum as presented in Fig. 1*a*. Two light-reactions in photosynthesis are discussed in the literature[2,5,6,8].

Information about the difference spectrum of C/C^+ in the range of 400–440 mμ (in Fig. 1*a* masked by the absorption changes of cytochrome) and about the chemical nature of C/C^+ is given by experiments with aged chloroplasts of spinach. Vernon, Kamen and Zaugg[4] have shown that aged inactive chloroplasts can again reduce triphosphopyridine nucleotide (TPN) in light after addition of reduced substances (dichlorophenol-indophenol with ascorbic acid). We observed that after such additions (reduced phenazine methosulphate, with ascorbic acid in excess) the aged

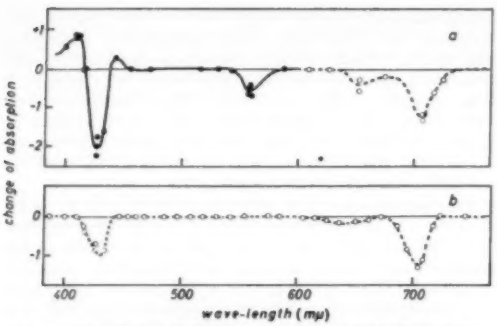

Fig. 1. *a*. Difference spectrum of the trapped products of photosynthesis in spinach chloroplasts at −150° C. *b*. Difference spectrum caused by illumination of aged chloroplasts with reduced phenazine methosulphate (PMS) at +20° C.

chloroplasts showed a difference spectrum depicted in Fig. 1*b*. The changes of absorption at 430 and 703 mμ are both caused by one and the same substance *C*. This follows from the identical behaviour of the changes at 430 and 703 mμ at different physical and chemical parameters. For example, with increased concentration of reduced phenazine methosulphate (red.PMS) the decay-time of the absorption changes at 430 and 703 mμ decreases from approximately 10^{-2} sec. at 10^{-6} *M* to 10^{-4} sec. at 10^{-4} *M*. This means in addition that *C* is oxidized by light: $C \xrightarrow{h\nu_1} C^+$, and that C^+ is reduced by red.PMS in a dark reaction: $C^+ \xrightarrow[\text{PMS}]{\text{red.}} C$. With the addition of reduced dichlorophenol-indophenol (red.In.) the decay-time at 430 and 703 mμ also decreases but only to a limited value of 10^{-2} to 10^{-3} sec. depending on the condition of the chloroplasts. This means that red.In., contrary to red.PMS, does not react directly with C^+ but via an intermediate reaction (with cytochrome, see later) with a duration of 10^{-2} to 10^{-3} sec. By addition of different mixtures of ferro/ferricyanide it was possible to change the steady-state concentration of C/C^+. This can be seen by the dependency of the size of the light-induced absorption changes at 430 and 703 mμ on the ratio of ferro/ferricyanide. From such measurements we have estimated the redox potential of C/C^+ as $+ 0{\cdot}45 \mp 0{\cdot}01$ V. at *p*H 6 and 8. Kok[5] has discovered and already reported in detail about the changes at 703 mμ and +20° C. He found that the unknown substance causing these changes has a redox potential of +0·46 V. and can be oxidized by light at 710 mμ and reduced by shorter wave-lengths. Our measurements at 703 mμ and + 20° C. confirm his results. Our additional observation that in chloroplasts capable of reducing TPN the oxidation of *C* causes, apart from the changes at 703 mμ, a pronounced change at 430 mμ (see also ref. 3) leads us to suggest that the difference spectrum shown in Fig. 1*b* represents the oxidation of a chlorophyll-*a*. Besides type 0 and type 1 (see ref. 2) this is the third chlorophyll reaction observable in plants.

By exciting *Chlorella* with 'flashing light' at +20° C. it follows from the time of the changes of absorption (estimated at 703 mμ) that $C \longrightarrow C^+$ reacts within $< 10^{-5}$ sec. The decay time of the changes, that is, the duration of the back reaction $C^+ \longrightarrow C$ in *Chlorella*, has been estimated at 430 mμ as approx. 10^{-3} sec. Contrary to the strong temperature-dependent life-time ($\tau \approx 10^{-2}$ sec.) of the absorption changes at 475 and 515 mμ (see ref. 2)—caused by an enzymic back-reaction (E) of a reduced substance $X^- \longrightarrow X$—the back-reaction of $C^+ \longrightarrow C$ is virtually independent of temperature (0–40° C.).

Previous work[2] showed the time of cytochrome oxidation Cyt$\longrightarrow$Cyt$^+$ at 20° C. and 420 mμ to be 10^{-3} sec. This value is falsified by the absorption changes of $C \longrightarrow C^+$, which, as now can be seen, also take place at 420 mμ. New measurements of the time of increase of Cyt $\longrightarrow$ Cyt$^+$ at 405 mμ give a temperature-independent value of about 10^{-2} sec. This value corresponds to the decay-time of $C^+ \longrightarrow C$.

The rapid increase of $C \longrightarrow C^+$ (less than 10^{-5} sec.), the trapping of C^+ and Cyt$^+$ at −150° C., the production of C^+ and Cyt$^+$ in one and the same light reaction $h\nu_1$, the identification of *C* as chlorophyll-*a* and the fact that the decay-time of C^+ corresponds to the time of increase of Cyt$^+$ gave us the idea that the cycle of C/C^+ is the chlorophyll reaction operating between cycles 1 and 2 in Fig. 2 of ref. 2. This led to an extended reaction pattern shown in Fig. 2. The trapping of both C^+ and Cyt$^+$ at −150° C. is probably caused by a charge compensation at −150° C. between C^+ and Cyt: $C^+ + \text{Cyt} \rightleftharpoons C + \text{Cyt}^+$. This is further evidence for the direct connexion of these substances.

The energy difference between the cycles provides evidence that phosphorylation is generated by the energy released between cycles 3 and 4 (see ref. 2). Losada *et al.*[4] suggest phosphorylation between chlorophyll and cytochrome.

As aged chloroplasts with red.PMS show only changes of absorption at 430 mμ and 703 mμ (Fig. 1*b*) it follows from Fig. 2 that this system only operates with cycles 1 and 2 and is the most simple and stable photo-active unit in plants. (C^+ reacts only with PMS in aged chloroplasts; in fresh chloroplasts C^+ is shielded from the attack of PMS.) Addition of *p*-chlorophenyl-dimethylurea (CMU) or phenanthroline (strong poisons for oxygen production) have no influence on the absorption changes at 430 and 703 mμ in this simple system; this is in accordance with our scheme. Aged chloroplasts with small amounts of red.In. show absorption changes at 430 and 703 mμ and the difference spectrum of cytochrome (Fig. 1*a*). From Fig. 2 it follows, therefore, that in this case cycles 1, 2, and 3 are operating (Cyt$^+$ is reduced by red.In.). In fresh chloroplasts with CMU or with phenanthroline, oxygen production is stopped, and

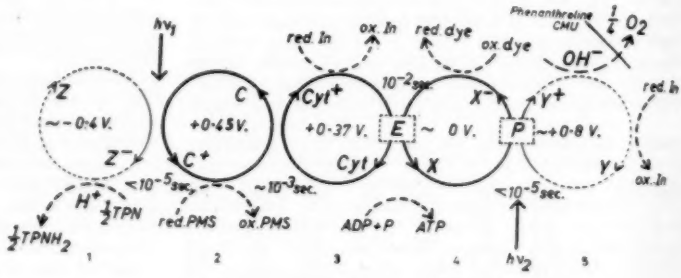

Fig. 2. Reaction pattern of photosynthesis

no change of absorption can be observed. By addition of red.In., however, all changes of absorption, as well as those caused by X/X^-, reappear. Therefore, in this case, cycles 1, 2, 3, 4 and 5 are operating and Y^+ must be reduced by red.In. instead of OH^- (Fig. 2).

Losada *et al.*[6] have assumed that in fresh chloroplasts with CMU and red.In. one light reaction was separated by blocking the other one with CMU. This cannot have been the case, since from the results reported here it follows that addition of CMU (blocking the production of oxygen by poisoning the reaction of Y^+ and OH^-) has together with red.In. no effect on either light reaction, because Y^+ can react with red.In. instead of water (OH^-).

Note added in proof. Recently Kok[7] has reported changes in absorption at 432 mμ in inactive chloroplasts extracted with acetone. He tends to ascribe these changes to those at 703 mμ and assumes tentatively that both changes are caused by a chlorophyll-*a*.

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New Ninhydrin-Reactive Substance from Red Algae

During an investigation on the free amino-acids in marine algae, I isolated a new ninhydrin-reactive substance from three species of red algae *Chondrus ocellatus*, *Neodelsea yendoana* and *Iridaea cornucopiae* by the use of anionic ion-exchange resin 'Amberlite *IR*-45' and 'Dowex 2'. This substance gave an empirical formula $C_5H_{11}O_5NS$ and melted at 258° C. Optical rotation gave a value $[\alpha]_D^{13} = -1{\cdot}15$ ($c = 5$, *M* sodium hydroxide solution). It was not very sensitive to ninhydrin, but gave a bright brown colour with Plattner's reagent. Titration with 0·1 *N* sodium hydroxide, in alcoholic solution, gave a value 63·80 per cent (calculated as $C_4H_7N—SO_3H—COOH$, 66·16 per cent). By the method of Kuhn and Roth, at least one radical, $C—CH_3$, was estimated. The oxidation products obtained, after treatment with potassium permanganate solution, were taurine, alanine and propionic acid. From these results, the structure *N*-(1-carboxy-ethyl)-taurine, that is ($HO_3S—CH_2—CH_2—NH—\underset{\displaystyle CH_3}{\underset{|}{CH}}—COOH$), was assumed. Crystals obtained from α-brompropionic acid and taurine by chemical synthesis corresponded well with the isolated substance not only in melting point but also in R_F values on various paper chromatograms.

Further investigations on the non-absorbable fraction of algal extracts on strong cationic ion-exchange resin revealed the wide distribution of this substance with taurine in red algae. The only exception was in the case of *Chondria crassicaulis*, from which a sulphur-containing amino-acid, 1,4-thiazane-3-carboxylic acid *S*-oxide had previously been isolated[1]. Not a trace of this substance could be detected here. Furthermore, they have not been detected so far in brown or green algae.

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Sex Hormones as a Possible Factor controlling Biosynthesis of Arylesterase

Blood plasma arylesterase in pigs is controlled genetically by a set of alleles, each determining a plasma arylesterase activity of 0, 25, 50, 75 and 100[1]. The distinguishable phenotypes are the manifestation of an additive effect of a pair of these alleles. The plasma of new-born piglets has no arylesterase activity, and differentiation into groups of various esterase activities begins on the second to fourth day of life, adult values being reached at the age of about 50 days. There are no sex differences for the biosynthesis of this enzyme, which, however, is influenced by sex hormones, as will be briefly reported here.

In the genetical work, the male piglets were castrated at the age of three to four weeks. The adult arylesterase activity values of these boars were fairly constant. However, certain male piglets were selected for use in later cross-tests. With these uncastrated boars, it was observed that arylesterase activity after maturity was much lower than before sexual maturity[2]. For example, it was demonstrated that with a boar of esterase activity (phenotype) 150 before maturity, the activity gradually decreased with increasing age to a value of about 30, and that this low phenotypic activity was then fairly constant. This phenomenon was found to be common to all mature boars, the low phenotypic esterase activity of which had no influence on expected segregation in the progeny. Evidence has now been presented that this decrease in arylesterase activity is due to the influence of male sex hormones on the biosynthesis of active protein. When a mature boar with a low phenotypic activity of 30 was castrated (Fig. 1*B*) the arylesterase activity increased gradually and had reached after 3 weeks a value (120–140) close to that found before maturity (150). Moreover, this high activity could again be reduced to the low value when 100–250 mgm. of testosterone propionate was administered daily intramuscularly. The low value was reached about three weeks after hormone treatment began. Testosterone had no effect on the enzyme *in vitro*.

This observation has been confirmed with boars of various genotypes. Testosterone also enhanced the decrease in arylesterase activity when administered shortly after maturity (Fig. 1*A*). Female sex hormones (for example, œstradiol) had no effect on the biosynthesis of this esterase. It is possible, however, that in addition to testosterone other agents (for example, the adrenocortical hormones) are also engaged in the regulation of plasma arylesterase activity in boars.

In contrast to mature boars, sows show small normal variations in plasma arylesterase activity.

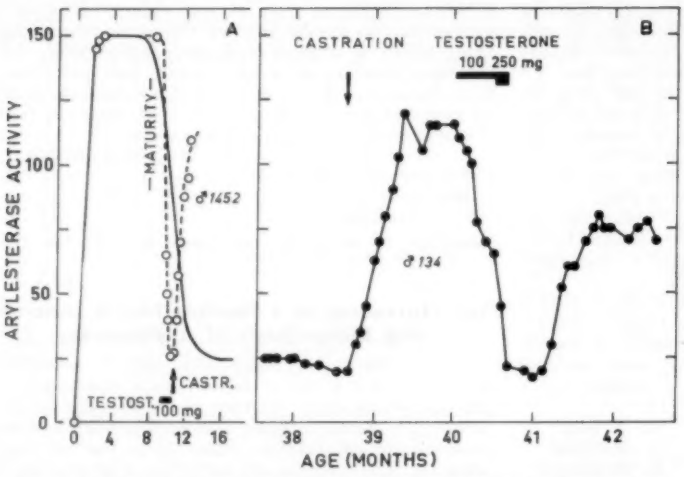

Fig. 1. Effect of castration and testosterone propionate on plasma arylesterase activity of boars *in vivo*; the hormone was administered intramuscularly daily as indicated. Esterase activity expressed in b_{30}/0·02 ml. plasma with phenyl acetate as substrate (Warburg technique). *A*, solid line, variation in plasma arylesterase activity of boars from birth to adult age (generalization); broken line and open circles; effect of testosterone shortly after maturity and before castration of boar 1452 (phenotype 150; weight 140 kgm.). *B*, effect of testosterone after castration of boar 134 (phenotype 150; weight 250 kgm.)

In some cases, sows had higher esterase activity when sexually matured than two to three months before maturity. It was observed that the activity was slightly higher shortly before partus, decreased below the normal value at the time of delivery, increased during 2–3 weeks after delivery and then again decreased to normal. It is possible that these variations in activity are partly due to changes in volume of blood during pregnancy. Œstradiol had no effect on the esterase activity in mature sows. Testosterone had an opposite effect on this activity in sows compared with boars, that is, increase in esterase activity which, however, was not so marked as the decrease in boars.

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A New Unsaturated Uronide isolated from Alginase Hydrolysate

Alginase, which brings about the enzymic hydrolysis of alginic acid, has been found in the organs of a few of the lower animals[1,2] and in some kinds of bacteria[3,4].

According to Kooiman[5], and others[3,4], the enzyme of the bacteria can split alginic acid into smaller units and mannuronic acid monomers. Recently, Eller and Payne[6] isolated a bacterium, *Alginomonas alginica*, which can oxidize mannuronic acid, but experiments concerning the isolation of the intermediates have not been reported. During work on alginase hydrolysates we found a new type of mannuronide, containing an unsaturated sugar acid and a mannuronic acid; the compound is the main component of the breakdown products of alginic acid. The isolation and properties of this new compound are described here.

One part of homogenized liver of abalone (*Haliotis discus hannai*) was mixed with 4 parts of distilled water at 4° C. After 2 hr. the mixture was centrifuged, and the supernatant filtered through pulp. The clear water extract which was obtained was used as the enzyme solution. 7·5 litres of 1 per cent sodium alginate solution were incubated with 750 ml. of the enzyme solution at 30° C. The *p*H value of the reaction mixture was adjusted to 7·0 with dilute sodium hydroxide during the course of the reaction. Progress of the enzyme reaction was followed by noting the increase in reducing power, which was measured by the iodometric method of Somogyi[7]. After 20 hr. the reaction was stopped by the addition of sulphuric acid. The precipitate was filtered off, and the filtrate neutralized with barium carbonate, re-filtered, and evaporated under reduced pressure below 40° C. About 300 ml. of the concentrate were passed through 'Amberlite *IR*-120'. The neutralization with barium carbonate, filtration, and resin treatment were repeated, and the eluates and washing water combined. About 500 ml. of the solution were put on a carbon–'Celite' column, 11 × 9 cm., and eluted with water, 10 per cent ethanol, and then 30 per cent ethanol. The eluates were collected in 500-ml. amounts at a flow-rate of 4 ml./min.

The course of the separation on the column was checked by submitting the concentrate taken from each fraction to ascending paper chromatography in the systems: *n*-butanol/acetic acid/water (5 : 2 : 3) and pyridine / ethyl acetate / acetic acid / water (5 : 5 : 1 : 3). According to the results of the chromatograms, individual fractions obtained from the column were pooled. As the water fractions between 3·5 and 25 l. were observed to contain one main product of hydrolysis, they were combined, evaporated under reduced pressure below 40° C., and lyophilized. Colourless needle-like crystals were isolated, with an approximate yield of 7·5 gm. The crude product was recrystallized from water and had a melting point of 135·5–136·5° C. (decomposed). (Calculated for $C_{12}H_{16}O_{12}.H_2O$; C, 38·92, H, 4·86; found; C, 38·98, H, 5·22 per cent.) An infra-red spectrum of this compound in potassium bromide showed two carboxylic groups. The molecular weight was 400 ± 40 (Barger's method). This compound showed a strong absorption peak in the ultra-violet at 232 mμ (in water) with $E^{1\%}_{1cm.}$ of 143·0, which disappeared on bromination or palladium hydrogenation, indicating the presence of an α,β-unsaturated acid[8]. On paper chromatograms, significant differences between this and the brominated or palladium-hydrogenated compound were observed. On hydrolysis with sulphuric acid at 100° C. for 1 hr., the

spots on chromatograms of the hydrolysates showed the characteristics of mannuronic acid and of mannuronic acid lactone, from which the occurrence of the mannuronic acid moiety in the compound can be inferred. The colour reactions of this compound are shown in Table 1.

Table 1. Colour Reactions of the Unsaturated Uronide

Reagent	Maximum absorption ($m\mu$) or colour tone	Remarks
Carbazole*	530	Same as hexuronic acid
Phenol-H_2SO_4†	480	On bromination shows λ_{max} 480 and 560 $m\mu$
Thiobarbituric acid‡	550	Same as pectin transeliminase hydrolysate§
Aniline hydrogen phthalate	brownish red	Same as hexuronic acid
o-Aminophenol	Yellow⟶brown	Differs from hexuronic acid which shows blue

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From these results it can be seen that the structure of this new type of uronide may be a diuronide containing $\Delta^{4,5}$-mannuronic acid and the mechanism of the enzyme action seems to involve the splitting of the glycosidic bond by an elimination reaction, thereby introducing unsaturation into the liberated molecules, as already seen in the splitting mechanisms of hyaluronic acid[8] and pectin[9].

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Determination of the Amino-Acid Composition of Bovine Growth Hormone

The amino-acid composition of bovine growth hormone has been determined[1] by Levy's method[2]. This procedure, which involves paper chromatography of dinitrophenyl derivatives of amino-acids, is subject to certain limitations such as variable recoveries of tyrosine and lack of resolution of the leucine derivatives. The development of automated ion-exchange chromatography of amino-acids[3] has prompted a re-investigation of the amino-acid composition of this protein.

All the samples of bovine growth hormone employed in this work were furnished by Dr. C. H. Li and were prepared by a previously described method[4]. Determination of the nitrogen content of the anhydrous, ash-free protein by the Dumas method has given a value of 16·7 per cent in contrast to that of 15·8 per cent reported elsewhere[1]. This value was confirmed by the amino-acid composition reported here and also by an independent analysis performed by the Elek Micro Analytical Laboratories, Los Angeles, California.

Hydrolyses were carried out for 24 or 72 hr. at 110° C. in a sealed evacuated tube. Approximately 4 mgm. of protein was transferred to the tube in a weighing tube followed by 10 ml. of constant-boiling hydrochloric acid redistilled in an all-glass system. After hydrolysis, acid was removed by repeated evaporation; the hydrolysate was then diluted to 10 ml. with 0·2 *N* citrate buffer, *p*H 2·2. Chromatograms of each hydrolysate were run in triplicate on a Spinco model *MS* amino-acid analyser.

Table 1 gives the detailed amino-acid analysis of one bovine growth hormone sample, preparation III. Appropriate corrections have been made for hydrolytic destruction. Including the tryptophan and amide values of Li and Chung[1], it will be seen that the analysis will account for the weight of the protein and the nitrogen content. A summary of the analyses for the three preparations is also shown in Table 1. Li and Chung's results[1] have been included for purposes of comparison. Preparation I is from the sample of growth hormone used for the original analysis of Li and Chung. It will be seen that the agreement is generally good. There are two discrepancies worthy of note. The total number of leucine and isoleucine residues obtained in the present analysis ranges from 62 to 68, the higher figure being found in two of the three preparations. The older figure of 76 residues would appear to be subject to question. In this work significantly higher values for serine have been obtained. It is possible that this reflects a shortcoming in the Levy procedure. The hydrolysis conditions might also be different to the extent that

Table 1. Amino-Acid Analyses of Bovine Growth Hormone

	Preparation III				Summary (residues)		
	Gm. amino-acid residues/100 gm. protein	Deviation*	N as percentage of total N	Residues 45,652 m.w.	Preparation I	Preparation II	Li and Chung (ref. 1)
Lys	6·20	0·5	8·02	22·1	23	23	23
His	2·03	1·4	3·68	6·8	7	7	7
Arg	8·20	1·1	17·39	24·0	26	26	26
Asp	9·06	0·6	6·52	35·9	33	34	35
Thr	4·70	0·8	4·67	25·7	24	25	26
Ser	5·26	1·2	5·01	27·6	25	27	22
Glu	14·03	1·3	9·01	49·6	51	52	50
Pro	3·21	4·3	2·74	15·1	13	14	14
Gly	2·84	0·7	4·12	22·7	22	22	20
Ala	4·61	0·7	5·38	29·6	29	30	31
CyS/2	1·80	5·0	1·35	8·1	8	8	8
Val	3·02	2·4	2·53	13·9	14	14	14
Met	2·41	2·1	1·52	8·4	7	8	7
Ileu	3·27	0·9	2·40	13·2	14	14	76§
Leu	12·27	0·6	8·99	49·4	54	54	
Tyr	4·26	1·4	2·16	11·9	12	13	11†
Phe	8·15	0·7	4·59	25·3	25	26	27
Tryp	1·25‡		1·13	3·1			3
NH_3			6·50‡				
	97·57		97·71	392·4	387	397	400

* Mean deviation as ± per cent of mean.
† Léonis and Li (ref. 6) report 12 residues.
‡ Taken from Li and Chung (ref. 1).
§ Sum of isoleucine and leucine residues.

different destruction factors have been obtained. Other differences may be indications of 'microheterogeneity'[5]. The tyrosine values are in good agreement with the spectrophotometric data of Léonis and Li[6] for bovine growth hormone. The chromatograms did not reveal the presence of any new or unusual amino-acids.

I wish to thank Dr. C. H. Li, of the University of California, for the gift of the growth hormone samples. Dr. S. H. Eppstein has contributed valuable advice during the course of this work. Microanalyses were performed by the Physical and Analytical Laboratory of the Upjohn Co. and the Elek Micro Analytical Laboratories, Los Angeles, California.

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Starch Gel Electrophoresis of Hen Egg White, Oviduct White, Yolk, Ova and Serum Proteins

The Smithies[1] starch gel technique has recently been successfully applied by Lush[2] to demonstrate genetic polymorphism in proteins of the white of a hen egg.

Lush[2] referred to two electrophoretically distinct components of crystalline hen ovalbumin described by me[3]. Three—and not two—components were reported in this work, which was later briefly reported[4]. The electrophoretic technique used in an examination of hen serum and egg proteins is now described in more detail. Suitable starch gels containing 15–17 per cent w/v starch were prepared from B.D.H. soluble starch and buffered as recommended by Smithies. The 'Perspex' troughs containing the starch gels were placed on a metal sheet in contact with an ice block in a cold room at 0°. The run was continued for 8 hr., at 400 V. and 10 m.amp.

The components of the 'thin', 'inner thin' and 'thick' layers[5] of hen egg white proteins were compared with those of the viscous fluid obtained from the magnum region of the oviducts of laying hens. The protein composition appeared to be identical by comparison of pairs of these fluids on a single gel in all four cases. Sixteen well-defined bands were observed (Fig. 1*a*). The fast anodic components 16 : 15 : 14 were shown to be identical with three components of a sample of five-times crystallized ovalbumin, $A_1 : A_2 : A_3$ respectively, as described by Cann[6].

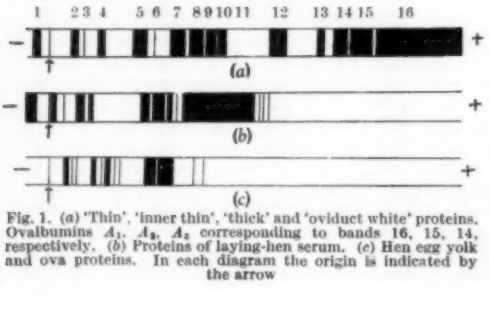

Fig. 1. (*a*) 'Thin', 'inner thin', 'thick' and 'oviduct white' proteins. Ovalbumins A_1, A_2, A_3 corresponding to bands 16, 15, 14, respectively. (*b*) Proteins of laying-hen serum. (*c*) Hen egg yolk and ova proteins. In each diagram the origin is indicated by the arrow

For comparative purposes the protein patterns of hen serum (Fig. 1*b*), egg yolk and ova isolated from the ovary (Fig. 1*c*) are given. The actual position of the bands in the figures cannot be directly compared since these patterns were obtained from different gels. It can be seen that the high resolving power of starch electrophoresis is well suited to the study of species differences in complex protein and lipoprotein mixtures.

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Electrophoretic Separation of Conjugated Bilirubin on Paper

In previous experiments[1] the 0·02 *M* phosphate buffer of *p*H 6·8 was used for electrophoresis of bile pigments on paper. Under these conditions (in the absence of proteins) the conjugated bilirubin migrating anodically separates from the unconjugated bilirubin which remains on the starting line. The presence of bile salts clearly influences this separation. Purified preparations[2] of conjugated bilirubin which are devoid of bile salts migrate in the form of a tail beginning just from the starting line. If bile salts are present a sharply defined zone of conjugated bilirubin separates, migrating in front of the bile salts. The velocity of migration of this zone in the presence of bile salts is considerably higher than the rate of migration of pure preparations of the pigment.

For example, the purified preparation, containing about 80 per cent of pigment II (as determined according to Billing[3]) after paper electrophoresis (1 m.amp./1 cm. of Whatman No. 1 paper, potential gradient about 12·5 V./cm., 2 hr. 15 min.) in 0·02 *M* phosphate of *p*H 6·8, yielded a diffuse zone beginning at the starting line and tailing to 9 cm. The same preparation in the presence of about 10-fold excess (on the weight basis) sodium taurocholate (and taurodeoxycholate) migrated simultaneously as a relatively narrow zone between 12 and 13 cm. from the start. The separation of both fractions of conjugated bilirubin (pigment I and pigment II[4]) could not be observed. Analogous results were obtained with the use of sodium acetate–acetic acid buffer of *p*H 5·2; only the rate of migration was slightly lower. Barbital buffer of *p*H 8·6 cannot be recommended owing to the instability of conjugated pigments in the alkaline range. The migration of unpurified preparations in this buffer proceeds in the same way as in phosphate buffers.

For the purpose of electrophoretic separation of purified preparations of conjugated bilirubin (that is, those which were devoid of excess bile salts) the systems containing pyridine were tested. Such systems were described by Grassmann *et al.*[5] (*p*H 3·9) and by Runeckles and Krotkov[6] (*p*H 6·0). Both yielded better results than mere aqueous solutions; systems containing more pyridine caused less tailing. The system (*p*H 6·1) was selected containing 5 ml. of glacial acetic acid, 70 ml. of pyridine in 1 litre. Under

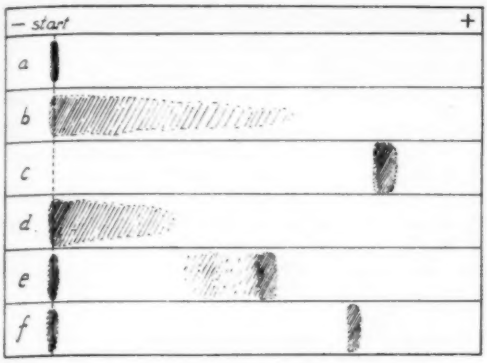

Fig. 1. Electrophoresis of bile pigments on filter paper. *a*, Unconjugated bilirubin in 0·02 *M* phosphate, *p*H 6·8; *b*, conjugated bilirubin (about 80 per cent of pigment II) in 0·02 *M* phosphate, *p*H 6·8; *c*, same in the presence of excess bile salts; *d*, same preparation as in *b* but in 0·08 *M* sodium acetate, *p*H 5·2; *e*, same preparation as in *b* with free bilirubin added, in pyridine acetate; *f*, same as in *e* in the presence of excess of bile salts

these conditions the separation of conjugated from unconjugated bilirubin was complete and both fractions of conjugated bilirubin were visible on the electrophoretograms if an appropriate amount of pigment was applied (corresponding to about 100 μgm. of bilirubin on 2–3 cm. width of paper). The presence of an excess of bile salts precludes the possibility of distinguishing between the two conjugated forms.

The use of buffers containing pyridine acetate enables the electrophoretic separation even of preparations devoid of bile salts, which was not possible in mere aqueous buffer solutions.

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Enzymatic Hydrolysis of Biliary Conjugates of 3,4-Benzpyrene on Filter Paper

Metabolites of numerous biologically active endogenous and exogenous organic compounds are excreted in bile and urine as glucuronic acid conjugates. In the course of studying the mechanism of action of carcinogenic polycyclic hydrocarbons, we wished to identify the metabolites of certain of these agents. For this purpose, ^{14}C-3,4-benzpyrene was injected into rats, and bile was collected by an indwelling cannula, utilizing a technique already described[1]. The bile fractions were collected in an automatic fraction collector, and enzymatic hydrolysis with β-glucuronidase was undertaken. Characterization of all the metabolites by this technique proved to be impossible because of the extreme instability of some of the hydrolysis products. We therefore attempted to determine whether the extent of oxidation and the class of conjugate formed could be established by direct analysis of the conjugates.

Two methods utilizing paper chromatography with subsequent enzymatic hydrolysis on paper were developed. Williams and Bevenue[2] have reported the use of a technique for the identification of raffinose and sucrose by enzymatic hydrolysis on paper chromatograms. In our work, migration was initially accomplished along one axis using a solvent system consisting of *n*-butanol/pyridine/water (3 : 2 : 2) for the separation of fluorescent compounds. If satisfactory separation resulted, the enzyme was applied to the individual spots, which were then incubated for 90 min. A second chromatographic separation of compounds in an axis perpendicular to the first was then done, using the same solvent system. Under these conditions, unhydrolysed conjugates were found lying on the diagonal across the paper while those undergoing hydrolysis were usually detected in greater proximity to the solvent front of the second migration.

When the number of metabolites was large or when separation in one dimension was unsatisfactory for further analysis, a two-dimensional chromatogram was prepared prior to application of the enzyme. The papers were then scanned for radioactivity in a two-dimensional scanner (Packard Instrument Co.), following which fluorescence spectroscopic analysis was used to characterize the metabolites. (In certain experiments, non-isotopic 3,4-benzpyrene and pyrene were used, and qualitative results were then obtained exclusively by fluorescence analysis.)

Following two-dimensional chromatography, using *n*-butanol/pyridine/water (3 : 2 : 2) as the first solvent system and *n*-butanol / acetic acid / water (4 : 1 : 5) as the second, the individual spots were identified by fluorescence, following which they were incubated for 90 min. with a solution containing 100 units of the enzyme. The spots were then cut out of the chromatogram and attached to a new paper strip for a third chromatographic separation for which either the original or the second solvent system was used. Excellent results were obtained by cutting two semi-circles in the fresh paper strip and slipping the cut-out spots into these slits in a manner which permitted satisfactory contact between the two papers. Following development of the chromatogram, the strip was analysed for fluorescent spectra and/or the presence of radioactive foci. By this technique data on the original compound, its conjugation, rate of hydrolysis, and stability and nature of the free parent substance were obtained. Wherever technically feasible, ultra-violet absorption spectroscopy was used in addition to fluorescence spectroscopy.

Basic information concerning the optimal conditions for enzymatic action on filter paper was obtained by studying the hydrolysis of phenolphthalein glucuronides (California Foundation for Biochemical Research) as suggested by Talalay *et al.*[3]. All work was carried out on Whatman No. 1 filter paper using 0·1 molar acetate buffer at *p*H 4·5. Time studies were performed in which a substrate containing 25 μgm. phenolphthalein as the glucuronide and 100 units of the enzyme at 37° C. were used. These were placed on a screen inside a still Dubnov incubator. These results are presented in Fig. 1, where they are further compared with results obtained following: (*a*) substitution of a test-tube for the filter paper; (*b*) changing of the incubation temperature to 25° C.;

(*c*) changing the concentration of phenolphthalein in the substrate to 5 μgm. It will be noted that, in the presence of 100 units of enzyme, 90 per cent hydrolysis occurred after 10 min. incubation at a concentration of 5 μgm. of substrate. In contrast, 60 per cent hydrolysis occurred at the five times greater concentration. As anticipated, a greater yield resulted from hydrolysis in a test-tube, and a lower yield was obtained when the temperature was reduced to 25° C.

Progressively increasing the enzyme concentration above 50 units per spot resulted in diminishing the rate of increase of hydrolysis, suggesting that 50 units of enzyme would provide an excess for all concentration ranges likely to be encountered (Fig. 2). The constant use of 100 units of enzyme in the presence of altered concentrations of the substrate resulted in a hydrolysis-rate of 100 per cent down to 60 per cent with the decreasing yield paralleling the increase in concentration of substrate (Fig. 3). At the concentration-range encountered in work on bile, that is, between 1 and 2 μgm. of metabolite, a yield of almost 100 per cent may properly be anticipated.

Our work has further established that hydrolysis does not occur if the enzyme is denatured, while little hydrolysis is noted when the strips are not perfectly free of solvent after chromatography. Hydrolysis was uniformly decreased in the presence of bile, and for this reason enzymatic hydrolysis was carried out only after chromatography in all cases. This emphasizes the need for enzymatic hydrolysis subsequent to chromatography. Hydrolysis of conjugates of oxidation products of benzpyrene does not occur as rapidly as the hydrolysis of phenolphthalein

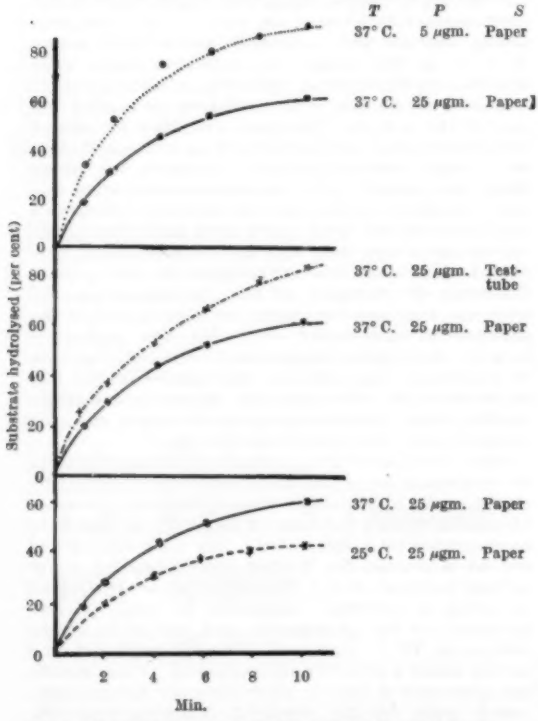
T P S
37° C. 5 μgm. Paper
37° C. 25 μgm. Paper
37° C. 25 μgm. Test-tube
37° C. 25 μgm. Paper
37° C. 25 μgm. Paper
25° C. 25 μgm. Paper
Substrate hydrolysed (per cent)
Min.

Fig. 1. Hydrolysis of phenolphthalein glucuronide on filter paper by β-glucuronidase. Effect of temperature (*T*), phenolphthalein glucuronide concentration (*P*) and site (*S*)

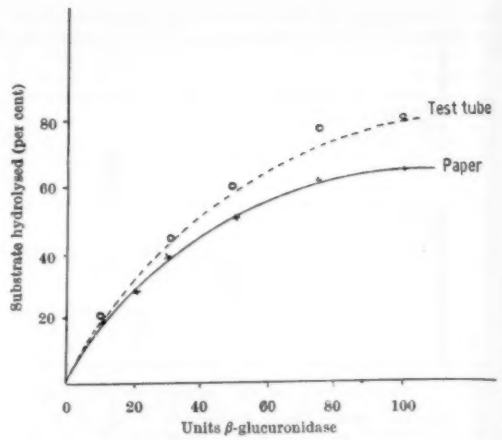

Fig. 2. Effect of enzyme concentration

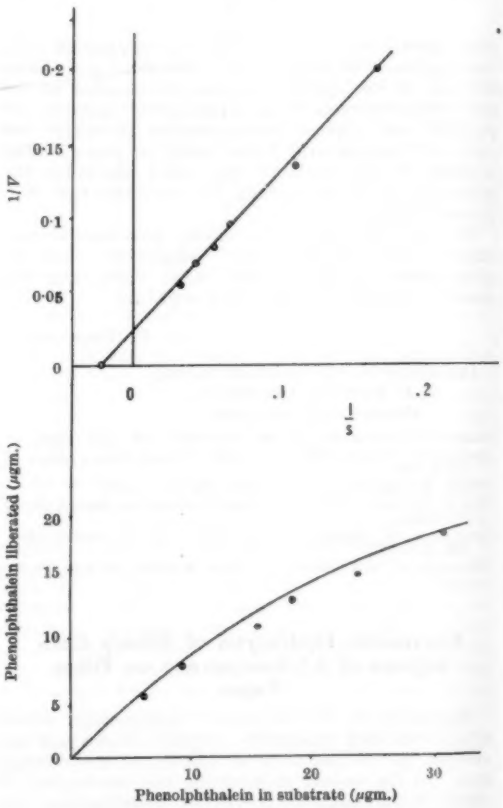

Fig. 3. Effect of substrate concentration

glucuronide, and therefore the hydrolysis time was increased to 90 min. in contrast to the 10 min. used for phenolphthalein. Detailed analysis of the biliary metabolites of polycyclic aromatic hydrocarbons will be presented in a subsequent publication.

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PHYSIOLOGY

Respiratory Chain Phosphorylation in Sub-Cellular Particles isolated from the Hepatopancreas of *Carcinus maenas*

In previous publications[1,2] I have shown that the respiratory chain present in respiring particles isolated from the hepatopancreas of *Carcinus maenas* (the common shore crab) contains pyridine nucleotides, flavoproteins and cytochromes c_1+c, b, a and a_3 in the proportions 19 : 3 : 1 : 1 : 1 : 1. All these respiratory pigments were shown to undergo oxidation and reduction during the oxidation of succinate, but the pyridine nucleotides reacted too slowly to be considered as a member of the respiratory chain associated with the oxidation of succinate. This communication presents evidence which shows that the oxidations catalysed by this respiratory chain are associated with the production of adenosine triphosphate.

The respiring particles used in these experiments were isolated by differential centrifugation of homogenates of hepatopancreas in 0·25 *M* sucrose[1]. The P/O (phosphorus to oxygen) ratios associated with the oxidation of succinate, fumarate and α-oxoglutarate were measured using Warburg respirometers and an inorganic phosphate uptake method. The respiring particles were suspended in a reaction mixture containing 5 m*M* substrate, 5 m*M* magnesium chloride, 1 m*M* adenosine triphosphate, 5 m*M* phosphate buffer, *p*H 7·4 (10 m*M* for α-oxoglutarate), and malonate in the concentrations shown in Table 1. Sucrose was used to adjust the tonicity to 0·25 osmolar. 0·9-ml. samples of the suspension of respiring particles were placed in the main compartment of a 6-ml. respirometer flask, 0·1 ml. of a 20 per cent potassium hydroxide solution was placed in the centre well and 0·1 ml. of a 0·25 *M* glucose solution containing 4 mgm. of crystalline hexokinase was in the side-arm of the flask. After a 7-min. equilibration period at 25° C. the hexokinase–glucose solution was mixed with the contents of the main compartment and the oxygen consumption measured. The reaction was stopped by the addition of 0·1 ml. of a 40 per cent trichloroacetic acid solution. The uptake of orthophosphate was determined as the difference between the orthophosphate contents of the experimental flasks and flasks to which the hexokinase was added after the trichloroacetic acid. Sumner's method[3] was used to assay orthophosphate. The results of these experiments are given in Table 1.

The mean value of the P/O ratios allied to the oxidation of 4·5 m*M* succinate is 2·04. This value lies between those reported for rat heart sarcosomes, P/O ratio = 0·95, and for rat liver mitochondria, P/O ratio = 2·17 (ref. 4). It should be noted that the P/O ratio associated with the oxidation of succinate by rat liver mitochondria is inflated by the phosphorylation associated with the further oxidation of the fumarate produced by the action of succinic acid dehydrogenase. This would not appear to be the case in the respiring hepatopancreas particles, since the mean value of the P/O ratio associated with the oxidation of fumarate, 2·06, is only fractionally higher than that for the oxidation of succinate. The P/O ratios for the oxidation of α-oxoglutarate by the respiring hepatopancreas particles is just lower than the value given by rat heart sarcosomes, P/O ratio = 2·80 ± 0·20 (10), in experiments where a similar method of assay to that described above was used (Beechey and Alcock, unpublished results). In experiments where the further oxidation of succinate was prevented by the addition of malonic acid, the P/O ratio associated with the oxidation of α-oxoglutarate was actually lowered. It was 2·45 in the presence of 10 m*M* malonic acid and 1·92 in the presence of 30 m*M* malonic acid. These results suggest that the malonic acid is acting as an uncoupling agent in this system.

Table 1. P/O RATIOS ASSOCIATED WITH THE OXIDATION OF VARIOUS SUBSTRATES BY THE RESPIRING HEPATOPANCREAS PARTICLES

Substrate	P/O ratio*	Qo_2 (μl./hr./mgm. protein)*
Succinate	2·04 ± 0·32 (11)	29·9 ± 4·8 (10)
Fumarate	2·06 ± 0·46 (6)	18·7 ± 4·5 (6)
α-Oxoglutarate	2·72 ± 0·23 (7)	29·0 ± 4·8 (7)
+ 10 m*M* malonate	2·35 and 2·55	14·8 and 14·7
+ 30 m*M* malonate	2·07 and 1·89	12·0 and 11·9

* Data given as mean ± standard deviation (number of determinations).

It was Loomis and Lipmann[5] who first demonstrated the uncoupling of respiratory chain phosphorylation from oxygen consumption by dinitrophenol. The effect of dinitrophenol on the coupled oxidation succinate and fumarate by the hepatopancreas is shown in Fig. 1. It can be seen that the complete uncoupling of phosphorylation from respiration occurs at concentrations of dinitrophenol greater than 60 μ*M*. Thus the sensitivity of the phosphorylating mechanisms in these hepatopancreas particles to dinitrophenol is of the same order as that of the corresponding systems in mammalian liver mitochondria[6] and heart sarcosomes[7].

The results presented here show that the oxidation of succinate, fumarate and α-oxoglutarate by these respiring hepatopancreas particles is associated with the production of adenosine triphosphate and also

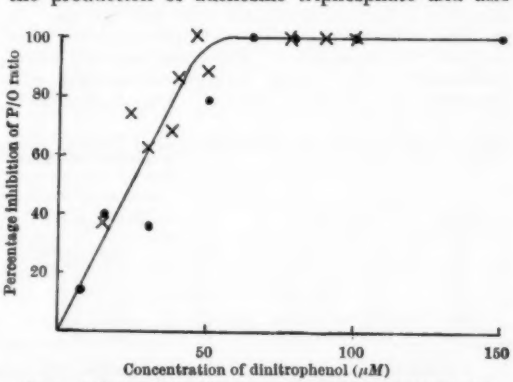

Fig. 1. Effect of dinitrophenol on the P/O ratio associated with the oxidation of succinate (●) and fumarate (×) by the respiring hepatopancreas particles

that the phosphorylating mechanism is uncoupled by dinitrophenol. The P/O ratios measured are quite similar to those given by mammalian mitochondria. These facts, together with the knowledge that these particles contain an active respiratory chain[1,2], suggest that respiratory chain phosphorylation occurs on these sub-cellular particles isolated from the hepatopancreas of *Carcinus maenas*.

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Growth Hormone Activity of the Anterior Pituitary Lobe of the Male Rat at Various Ages

Within the past few years several reports have dealt with the relationship between the age of an animal and the pituitary content of growth hormone. Pigs[1,2], ox[3], and the rat[4,5] have been examined. In general, it has been found that although the total amount of growth hormone in the pituitary gland increases greatly with age, the concentration of the hormone in the gland tends to remain fairly constant. The present work on the male rat supports this concept, but also indicates that relative to body-weight there may be a decreased amount of total growth hormone in the anterior lobe as the animal advances in age. This latter relationship has likewise been found in the larger species which have been examined, but previous evidence[5] on the rat has indicated a lack of correlation between the total pituitary growth hormone/body-weight ratio and age.

Male rats of the Long-Evans strain which were raised in the Bucknell University colony were used predominantly as the source of the pituitaries which were assayed. At ages of 10, 30, 60, 90, 180, 360 and 600 days the animals were killed by decapitation, the pituitary rapidly exposed, the posterior lobe removed and discarded, and the anterior lobe removed, weighed and placed in acetone. After 24 hr. in acetone the glands were air dried and placed in a vacuum desiccator until time for use. At the time of assay the desiccated anterior lobes were weighed, homogenized in saline, and injected intraperitoneally into young hypophysectomized rats prepared as indicated below. The homogenate, freshly prepared each day, was administered once daily for 4 days. With the exception of two of the age periods, the injections were given to assay animals at three dosage-levels, namely, 80, 280 and 720 μgm. of dry anterior pituitary a day. Parallel injections of standard growth hormone were given to another three groups of hypophysectomized animals at dosage-levels of one-tenth the amounts of homogenate used. Another group of animals received saline. The 10- and 30-day-old pituitaries were injected at two levels only: 80 and 280 μgm. per day for the 10-day-group, and 32 and 392 μgm. daily for the 30-day pituitaries. For each of these two ages the growth hormone standard was likewise given in amounts equal to one-tenth the homogenate used. Six to eight hypophysectomized rats were used at each dosage-level for all groups.

The recipient animals used for each assay were albino male rats obtained from a commercial source at 22–24 days of age, and hypophysectomized when they were 28 days old. At 40–42 days of age they were randomized into a number of groups and the four-day period of injection begun[6]. Rats which had gained more than 8 gm. or lost more than 3 gm. postoperatively were eliminated from the assay. The operated animals were maintained on calf meal, fresh bread, and 10 per cent glucose in their drinking water.

Twenty-four hours after the last injection the rats were killed by ether anæsthesia and the tibias removed and prepared for epiphyseal measurement according to the procedure of Evans, Greenspan *et al.*[7,8]. Statistics were calculated according to methods given by Emmens[9].

It can be seen in Table 1 that the concentration of growth hormone activity in the anterior pituitary gland showed little change with age, the ranges of the estimated potencies being essentially the same for all groups. In Table 1 are also given the anterior pituitary- and body-weights at the various ages examined; it will be noted that the ratio of dry (or wet) anterior pituitary-weight to body-weight exhibited a decrease with age. The ratio was highest at 10 days and then dropped to a somewhat constant value for the ages up to 3 months. A further decrease was observed at 6 months, and the still lower ratio seen at 12 months was maintained at 20 months of age. Thus, with a rather similar concentration of growth hormone in the pituitary at all the ages investigated, these decreasing pituitary-weight/body-weight ratios would indicate that in the anterior pituitary gland there is less total growth hormone per gram of body-weight as the male rat ages.

Solomon and Greep[5], using female rats, found that the growth hormone concentration in the whole

Table 1. The Relation of Age to the Growth Hormone Potency of the Anterior Pituitary Lobe and the Anterior Pituitary-weight/Body-weight Ratio in the Male Rat

Age (days)	Potency and range* (per cent)	N†	Anterior pituitary average weights		Average body-weights (gm.)	Ratio of dry pituitary-weight to body-weight: mgm. of pituitary per gm. rat (× 10^{-2})
			Wet (mgm.)	Dry (mgm.)‡		
10	11·3, 8·2–15·6	162	1·20 ± 0·03§	0·19	21 ± 0·3§	9·05
30	15·8, 7·1–35·3	26	3·76 ± 0·04¶	0·60	84 ± 1·8¶	7·14
60	19·8, 9·0–44·0	31	7·5 ± 0·1¶	1·42	191 ± 3¶	7·43
90	13·3, 9·4–19·0	24	9·2 ± 0·1¶	1·74	247 ± 4¶	7·04
180	14·0, 8·1–24·1	26	9·4 ± 0·3	1·85	332 ± 8¶	5·57
360	20·0, 10·5–36·5	25	10·0 ± 0·3	1·96	386 ± 8¶	5·08
600	17·2, 11·3–26·3	18	11·0 ± 0·5	2·04	401 ± 15	5·09

* Growth hormone potency of pituitary preparation and range of potency at 95 per cent significance level.
† Number of pituitaries and number of animals weighed.
‡ Desiccated pituitaries of a given age were weighed collectively, thus standard errors are not available.
§ Standard error of the mean.
¶ $P < 0.001$ that the difference between this value and that of any younger age group in this column is due to chance.

pituitary likewise fluctuated within only a small range as the animals aged. They observed in addition, however, that the weight of the whole pituitary continued to increase in an approximate proportion to the body-weight increments of the animals. Therefore the total amount of pituitary growth hormone relative to body-weight was calculated to change very little, and showed neither a regular decrease nor increase throughout the age range studied. Whether the apparent discrepancy between this latter observation and that of the present communication, in this regard, is due to the sex of the animal investigated or to anterior lobe and whole pituitary differences is not clear. In support of the present results, it has been reported in both male and female pigs[1,2] and in the cow[3] that the total amount of growth hormone in the anterior lobe shows a decline in relation to body-weight as these animals age.

It is recognized that the amount of hormone present in a gland may well not indicate the amount or rate of secretion, and information concerning blood-levels of, and tissue sensitivities to, growth hormone at various age periods would be of interest.

The bovine growth hormone used as the standard was a gift of the Endocrinology Study Section of the National Institutes of Health. This work was supported by National Science Foundation grant *G*–5069.

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Permeability of the Mammary Gland to Sodium-24, Potassium-42, Chlorine-36 and Iodine-131

Phosphorus-32 and calcium-45 appear in the blood of goats a few minutes after their administration through the teat[1,2]. The regulation of milk electrolyte content is little understood, and it seems necessary to examine the problem of udder permeability further in order to understand these problems better.

In the present investigation the permeability of the mammary gland to sodium-24, potassium-42, chlorine-36 and iodine-131 has been studied, using goats in the first half of lactation. Polyethylene glycol (mean mol. wt. 4,000), which does not penetrate the udder epithelium[3], was used as a reference substance. The disturbances in udder function, which often follow the injection of fluid through the teat, have been avoided so far as possible by reducing the injected amount of fluid. The injected solutes are rapidly and fairly evenly distributed throughout the whole milk volume of the injected udder half[3].

Half of an udder was cannulated and about 2 ml. physiological saline containing tracer (10–40 μc. of sodium-24, potassium-42 or iodine-131, 1–2 μc. of chlorine-36) and polyethylene glycol was slowly injected, usually 4 hr. after milking.

In the experiments with iodine-131, 5-ml. samples of milk were drawn at regular intervals, and the disappearance of iodide was examined by comparing the expansion of the iodide and polyethylene glycol spaces.

In the other experiments the udder was cannulated some time after the injection and the milk collected in fractions. When the milk ceased to flow spontaneously an additional amount of milk was obtained with oxytocin. The volume of milk still left in the udder was calculated from the amount of polyethylene glycol retained in the udder.

The radioactive isotopes were counted in a Friesecke and Hoepfner *FH* 49, using an ordinary Geiger–Müller-tube for sodium and iodide and a proportional counter for potassium and chloride. Polyethylene glycol was determined turbidimetrically[4].

The results from one experiment with iodine-131 are summarized in Table 1. Between the 4th and the 19th hr. of the experiment the volume of water in the milk, measured as the distribution volume of polyethylene glycol, expands from 95 to 145 ml. while the iodide space increases to nearly 5 litres during the same time.

Table 1

Time after injection (hr.)		4·5	6·3	8	9·8	11·3	19
Distribution volume (ml.)	Polyethylene glycol	95	98	102	120	126	145*
	Iodine-131	265	346	447	875	1,192	4,606

* The goat used in this experiment was in an advanced stage of lactation, which explains the low production of milk.

After the first 4·5 hr. 40 per cent of the dose was still present in the udder. This agrees well with the results from other experiments with iodide.

The results of the experiments with sodium-24, potassium-42 and chlorine-36 are given in Table 2.

Table 2

	No. of experiments	Time between injection and sampling (hr.)	Activity remaining in the milk (per cent)
Sodium-24	2	1	13
Sodium-24	2	4	1
Potassium-42	3	4	36
Chlorine-36	6	4	37
Sodium-24* }	1	2	10
Chlorine-36* }			70

* Examined simultaneously in the same udder half.

It is evident that the sodium ions disappear far more rapidly than the others. The difference between sodium and chloride is especially well demonstrated in the experiment where they were examined simultaneously.

The ratio of the concentrations of the isotope and polyethylene glycol is usually lower in the last fractions of milk. Table 3 gives the results of one typical experiment with each isotope.

Table 3

Fraction No.	1	2	3	4	5	6	7	8
Sodium/PEG	38	33	30	27	29	7	7	
Potassium/PEG	21	20	19	18	16	16		
Chlorine/PEG	38	35	32	24	26	25	26	27

PEG, polyethylene glycol

Clearly the epithelium of the udder is fairly permeable to the ions examined, and Table 3 tends to show that this applies at least to the epithelium of the 'proximal' parts of the gland, that is, the finer ducts and the alveoli. More information is obviously

needed before definite conclusions can be drawn with regard to the processes involved in the permeation of these solutes. In view of the low sodium content of the milk the apparently high permeability of the udder with regard to sodium ions may, however, indicate participation of both diffusion and active transport of this ion.

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Particle-bound and Free Catecholamines in Dog Hearts and the Uptake of Injected Norepinephrine

Submicroscopic structures containing catecholamines have been found by high-speed centrifugation of homogenates or pressed juice of adrenal medulla[1–3], splenic nerves[4–6] and brain[7]. A granular fraction containing catecholamines has been isolated from the pressed juice of cyclostome hearts[8]. It was the purpose of this investigation to see whether also in mammalian hearts the catecholamines are stored in a particle-bound state; the question was also examined whether injected norepinephrine is taken up by submicroscopic cell elements. This is of interest since Raab and Gigee[9] reported a specific avidity of the heart muscle to absorb and store catecholamines.

Six puppy hearts, which have a high catecholamine content (Wegmann *et al.*, unpublished data), were submitted to successive low- and high-speed centrifugation after homogenization. The catecholamine content in the different fractions was analysed fluorimetrically. In another series of 7 animals an identical procedure was applied 20 min. after the intraperitoneal administration of 10 mgm. norepinephrine/kgm. The hearts were dissected into very small pieces in ice-cold physiological sodium chloride solution. After washing three times and re-suspending in ice-cold saline solution the hearts were homogenized in a Potter–Elvehjem glass homogenizer with a loose-fitting pestle. The homogenates were centrifuged at 600–1,000*g* for 10 min. The homogeneous supernatant was again centrifuged at 50,000–100,000*g* for 60 min. The sediment from this centrifugation was taken up in a few ml. of *N* hydrochloric acid. Both sediment and supernatant were then deproteinized by trichloracetic acid for fluorimetric estimation according to Euler and Lishajko[10] after adsorption on alumina and elution with acetic acid. Only a set of two filters (excitation at 412 mμ; emission at 508 mμ) was used; thus norepinephrine and epinephrine were determined together.

The catecholamine content in the different fractions of the normal hearts is shown in Table 1. The supernatant layer obtained by low-speed separation did not contain microscopically visible cell structures. After high-speed centrifugation 67–85 per cent (average 74·5 per cent) of the catecholamines were located in the small sediment.

The catecholamine content in the different fractions 20 min. after injection of 10 mgm./kgm. norepinephrine is shown in Table 2. In these experiments only 9–31 per cent (average 19·6 per cent) of the catecholamines were found in the sediment after high-speed centrifugation, the greater part being recovered in the supernatant. The difference between the results in the two series is highly significant ($P < 0.001$).

Table 1. Distribution of Catecholamines in Heart Homogenates of Puppies

Weight of heart (gm.)	Total homogenate (μgm./gm.)	Supernatant (800*g*) (μgm./gm.)	Supernatant (800*g*) Total homogenate (per cent)	Sediment (75,000*g*) (μgm./gm.)	Sediment (75,000*g*) Supernatant (per cent)
13	1·00	0·54	54	0·36	67
14	0·64	0·34	50	0·26	76
16	1·20	0·56	47	0·38	68
10·5	1·10	0·41	37	0·35	85
12·5	1·04	0·58	56	0·41	71
13·5	0·71	0·34	48	0·27	80
Mean	0·95	0·46	49·0	0·34	74·5

Table 2. Distribution of Catecholamines in Heart Homogenates of Puppies 20 min. after Intraperitoneal Injection of 10 mgm. Norepinephrine/kgm.

Weight of heart (gm.)	Total homogenate (μgm./gm.)	Supernatant (800*g*) (μgm./gm.)	Supernatant (800*g*) Total homogenate (per cent)	Sediment (75,000*g*) (μgm./gm.)	Sediment (75,000*g*) Supernatant (per cent)
12	2·15	1·16	54	0·36	31
11·5	12·2	10·33	85	0·93	9
10	4·00	2·64	66	0·78	30
7	3·76	1·83	49	0·39	21
11	2·75	1·44	52	0·41	29
12	3·90	2·59	67	0·34	13
18	13·3	10·92	82	0·42	4
Mean	6·01	4·42	65·0	0·52	19·6

This investigation demonstrates the presence of particle-bound catecholamines in dog hearts. More than 95 per cent of injected norepinephrine found in the supernatant after low-speed centrifugation were not particle-bound. Whether or not these large amounts recovered in the soluble fraction were intra- or extra-cellular cannot be stated at the present time.

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Response of the Reticulo-Endothelial System to Stimulation with Œstrogens

We have previously shown that diethylstilbœstrol and tri-*p*-anisylchloroethylene are strong stimulants of the reticulo-endothelial system[1]. We have also found that when the reticulo-endothelial system is stimulated by diethylstilbœstrol the body defence is raised, as indicated by increased phagocytic activity, elevation of the serum γ-globulin to about three times the normal level, increased antibody formation, and the protection of mice and guinea pigs against lethal doses of virulent bacteria. In previous experiments it was found that the minimum dose of diethylstilbœstrol required to stimulate the reticulo-endothelial phagocytes is about a hundred times greater than that necessary to induce œstrus[2] and that after treatment with 0·5 mgm. diethylstilbœstrol daily for 6 days the

phagocytic activity returns to normal about 8 days later[3].

The present work was carried out to ascertain: (1) the speed of action of diethylstilbœstrol and tri-*p*-anisylchloroethylene in stimulating the reticulo-endothelial system; (2) whether this stimulation is maintained during repeated treatment with diethylstilbœstrol.

In the following experiments the phagocytic activity was taken as the measure of stimulation of the reticulo-endothelial system; it was assessed by the rate of disappearance of a known amount of specially prepared carbon from the circulating blood, the method being a modification of that of Biozzi *et al.*[4]. The procedure used has been described in previous communications[5]. The total body phagocytic activity or phagocytic index is denoted by the symbol *K*.

Speed of action of diethylstilbœstrol and tri-p-anisylchloroethylene: Fifty-five male white mice (*T.O.* Swiss strain) of 20–25 gm. body-weight were used in this experiment. Fifteen animals each received 0·1 mgm. diethylstilbœstrol in 0·1 ml. arachis oil subcutaneously once daily; fifteen received 0·5 mgm. diethylstilbœstrol in 0·1 ml. arachis oil subcutaneously once daily; and a further fifteen received 1 mgm. tri-*p*-anisylchloroethylene in 0·2 ml. olive oil orally once daily. On the first, second and third day after the commencement of treatment with each compound the phagocytic activity was measured in groups of 5 animals by the carbon method referred to above. Ten animals were used as controls and received no treatment.

The results are shown in Table 1. The control animals showed a phagocytic index of 15 ± 2. Compared with the control values it can be seen that on the first day after the commencement of treatment with each of the compounds there is slight stimulation of the reticulo-endothelial system; by the second day there is moderate stimulation, and by the third day the system has become strongly stimulated.

Effect of repeated treatment with diethylstilbœstrol. Forty male white mice (*T.O.* Swiss strain) of 20–25 gm. body-weight were used in this experiment. Ten of the animals were used as controls and received no treatment. The remaining 30 animals were divided into three groups of 10 and were treated as follows: Group *A*, 10 animals each received 0·5 mgm. diethylstilbœstrol in 0·1 ml. arachis oil subcutaneously once daily for 6 days. On the eighth day after the commencement of treatment the phagocytic activity was measured in each animal by the carbon method, and the animals were then killed. Group *B*, 10 animals were given diethylstilbœstrol for 6 days, as described above. They were then left for 3 weeks, then given a second course of diethylstilbœstrol for 6 days; on the following day the phagocytic activity was measured in each animal, then the animals were killed. Group *C*, 10 animals were given a similar course of diethylstilbœstrol for 6 days. They were left for 3 weeks then given a second course of diethylstilbœstrol for 6 days; they were now left for a further 3 weeks then given a third course of diethylstilbœstrol for 6 days. On the following day the phagocytic activity was measured in each animal and then the animals were killed.

Table 1. Speed of Action of Diethylstilbœstrol and Tri-p-anisylchloroethylene in Stimulating the Reticulo-endothelial System

Compound used	Daily dose	Day after treatment commenced	Mean phagocytic index (*K* value) ± *S.E.*
Diethylstilbœstrol	0·1 mgm. in 0·1 ml. arachis oil subcutaneously	First	18 ± 1
		Second	37 ± 4
		Third	40 ± 1
Diethylstilbœstrol	0·5 mgm. in 0·1 ml. arachis oil subcutaneously	First	19 ± 1
		Second	33 ± 7
		Third	72 ± 4
Tri-p-anisyl-chloroethylene	1·0 mgm. in 0·2 ml. olive oil orally	First	25 ± 1
		Second	37 ± 2
		Third	60 ± 4
Controls			15 ± 2

Table 2. Effect of Repeated Treatment with Diethylstilbœstrol on Phagocytic Activity of Reticulo-endothelial System

Animal group	Mean phagocytic index (*K* value) ± *S.E.*
Group *A*	66 ± 3
Group *B*	58 ± 1
Group *C*	77 ± 1
Controls	16 ± 1

The results are shown in Table 2. The control animals showed a phagocytic index (*K* value) of 16 ± 1. Compared with the controls it can be seen that the total phagocytic activity or phagocytic index *K* remains high in all three experimental groups *A*, *B* and *C* and that the phagocytes continue to respond to stimulation during repeated treatment with diethylstilbœstrol.

The above results show that œstrogen treatment produces strong stimulation of reticulo-endothelial activity by the third day after the administration of the drug is commenced. When the treatment is stopped the stimulative effect lasts for about 1 week, during which it gradually diminishes, and by the eighth or ninth day the phagocytic activity has returned to normal[3]. These findings may be of clinical interest since they indicate the short period during which œstrogens need be used to raise the body defence in acute illness. The results also indicate that the reticulo-endothelial phagocytes continue to respond to stimulation during repeated courses of treatment with diethylstilbœstrol over a period of at least 9 weeks.

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Production of Aortic Occlusion resembling Acute Aortic Embolism Syndrome in Cats

A syndrome of acute aortic embolism occurring in cats resembles the condition found in man. An embolus, usually fragmented from an intracardiac thrombus, lodges at the iliac bifurcation, disrupting blood supply to the rear limbs. The symptoms include: paraplegia, loss of femoral pulse, cold rear extremities, initial muscular tenseness of the rear limbs followed by atrophy, and pain. In an effort to reproduce the signs of aortic embolism or 'saddle embolus', the posterior aortas of thirteen cats were occluded by various methods. The work here suggests that the blood-clot occluding the aorta is responsible for the hind-limb paralysis, accomplishing this

by some means other than mechanical circulatory blockage.

Experimental aortic ligations have been performed frequently on laboratory animals, many being concerned with occlusion of the aorta above the renal arteries[1,2]. According to Adams[3], suprarenal aortic occlusion causes paralysis by ischæmic damage to the spinal cord, whereas infrarenal aortic occlusion accomplishes this by peripheral nerve damage. Giuseffi[4] states that infrarenal aortic occlusion of more than 4 hr. is apt to produce paraplegia in the dog. In his work on dogs, Brooks[5] reports that there is a constant marked disturbance in function of the posterior extremities following ligation of the aorta between the inferior mesenteric and external iliac arteries.

Long-term infrarenal aortic ligation work on the cat is not readily found in the literature. Because clots were found at the iliac bifurcation in naturally occurring cases, these experiments were designed to look into the effects of low aortic ligation on symptomatology referable to the rear limbs.

Cats between the ages of 7 months and 5 years were divided into 3 groups according to the surgical procedure used on each. A pre-surgical aortogram was performed on every cat to determine patency of the aorta. Braided stainless steel wire or heavy silk was used as the ligature material.

Group 1 consisted of five cats in which a single ligature was placed around the aorta at the level of the iliac bifurcation. Complete occlusion of the aorta was determined by aortography immediately after surgery. From the day following ligation until they were killed one month later, these cats showed no symptoms of paraplegia. Subsequent aortograms demonstrated a progressive increase in collateral circulation at the ligation site. Although no femoral pulse could be palpated post-surgically, the rear legs remained warm.

Group 2 consisted of five cats which were ligated as above with a second ligature placed approximately 1·5 cm. proximal to the first ligature. In most cases the circumflex iliac arteries were also ligated since they were the only vessels emerging from the aorta between the two aortic ligatures. As in Group 1, these cats demonstrated no symptoms of paraplegia. The purpose of the double ligation was to determine whether or not a 1·5 cm. 'dead space' in the aorta would cause a slower build-up of collateral circulation than a single ligature. Results did not differ from Group 1.

Group 3, in which a clot was produced in the posterior aorta of three cats, was done in order to simulate the naturally occurring syndrome of aortic embolism. A permanent ligature was placed around the aorta at the iliac bifurcation as in Group 1. The circumflex iliac arteries were obliterated as in Group 2. A length of umbilical tape was then placed around the aorta 1·5 cm. above the first ligature to occlude the aorta temporarily. When the tape was drawn snugly, blood was trapped between the ligatures. 10–20 units of bovine thrombin (about 0·2 c.c. of fluid) was injected through a 27-gauge needle into the lumen of the aorta between the ligatures. After allowing 5 min. for a clot to form, the temporary tape ligature was removed. On the day after surgery all three cats were paralysed in the rear limbs. Sensory and motor functions were lacking. There was no femoral pulse and the rear limbs were cold to the touch. Muscle atrophy of the rear limbs was extensive at the end of one week. Aortography showed no increase in collateral circulation. After ten days of unabated paraplegia, all three cats were killed. In contradistinction to Groups 1 and 2, necropsy showed a small, ragged clot extending approximately 1 cm. above the ligature at the iliac bifurcation. The muscles of the rear limbs were pale in colour. There were no gross lesions of the spinal cord or peripheral nerves to the rear limbs.

The following conclusions are drawn from this preliminary study: (1) ligation of the aorta of the cat at the iliac bifurcation has no demonstrable effect on locomotion in spite of the diminution of femoral pulse, and (2) since the methods used in Groups 1 and 2 produced no paretic symptoms but the Group 3 method did, the clot apparently played some part in causing the paraplegia. Collateral circulation was evidently impeded in some way by the presence of the clot. It is doubtful whether this happened by mechanical blockage of collateral channels from the aorta since the clot extended up the aorta from the iliac bifurcation for only a short distance. In fact, the clot did not extend as far up the aorta as the proximal ligature in Group 2. The similarity of the symptoms of naturally occurring and experimentally produced aortic embolism is striking and leads us to believe that the mechanism involved in producing paraplegia is the same in both instances.

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RADIOBIOLOGY

Mast Cell Turn-over in Adult Mice

Mast cells are generally thought to be derived from undifferentiated mesenchymal cells and perhaps heteroplastically from lymphocytes, plasma cells or histiocytes[1]. Various conditions can cause mast cell granules to be discharged in a manner that has led some investigators to believe the cell is destroyed in the process. Yet Padawer[2] reported never having seen a mast cell in mitosis during his extensive investigation of these cells with colchicine and related substances. This does not preclude the possibility of mast cells being continually replaced by transformations from a population of proliferating precursor cells. Extensive mast cell degranulation occurs after administration of compound 48/80 and is followed within 4 days by the re-appearance of granulated mast cells[3]. This sequence of events was investigated radioautographically with sulphur-35 by Watson and Kennedy[4]. They observed sulphur-35 uptake within 24 hr. by 'ghost cells' which had a tissue distribution equivalent to that of the original granulated mast cells.

The question of mast cell turn-over has been investigated in the present experiment by using radioautography after repeated injections of tritiated thymidine because this method will detect cells synthesizing deoxyribonucleic acid (DNA) even if they represent only a very small fraction of the population, and because transformations to mast cells from a proliferating population of precursor cells would be detected through the gradual accumulation of labelled nuclei in the mast cell population.

Six adult, female, strain *A/Jax* mice were given 6 injections of 100 μc. tritiated thymidine at 4-hr. intervals and killed at 1, 2, 3, 4, 8, or 21 days after the initial injection. Tissues were fixed in Carnoy's fluid and processed for radioautography using a fluid emulsion method described elsewhere[5] with the modification that a 'Celloidin' coating over the sections was omitted. The radioautographs were developed after exposing for a month and then stained with aldehyde-fuchsin[6] or toluidin blue (0·01 per cent aqueous solution followed by air drying and immersion in 95 per cent alcohol) or hæmatoxylin and eosin. Mast cells and radioactive connective tissue cells were counted in one aldehyde–fuchsin-stained section from each time-interval for the three areas of connective tissue.

Table 1. Frequency of Radioactive and Non-radioactive Mast Cells and of Radioactive Cells from other Connective Tissue Cell Populations in Aldehyde–Fuchsin-stained Radioautographs

Tissue	Days after injection	Mast cells: Radio-active	Mast cells: Non-radioactive	Other cells: radio-active	Area (sq. mm.)
Skin	1	0	20	84	7·05
	2	0	21	38	3·37
	3	0	57	140	8·66
	4	0	77	112	7·92
	8	0	85	27	10·08
	21	0	26	23	2·44
Foot-pad	1	0	6	16	0·88
	2	0	10	54	2·70
	3	0	122	33	15·30
	4	0	8	7	0·83
	8	0	9	11	0·59
	21	1	110	11	5·50
Tongue	1	0	10	7	1·64
	2	1	64	6	5·23
	3	1	58	6	3·74
	4	0	8	12	5·51
	21	0	33	3	4·22
Totals		3	724	590	85·66

The use of aldehyde-fuchsin, which left the nuclei unstained, made it easy to identify silver grains over a nucleus, and facilitated the locating of mast cells by providing a specific stain for their cytoplasm. Slides stained with toluidin blue or hæmatoxylin and eosin were scanned as a further check on the mast cell population and to identify the various radioactive cells in the connective tissue. Counts of non-radioactive and radioactive mast cells and radioactive connective tissue cells in aldehyde–fuchsin-stained sections are given in Table 1. Radioactive mast cells were rare in these tissues, regardless of the time-interval. Radioactive cells were plentiful in other cell populations of the connective tissue: many radioactive fibrocytes, macrophages, neutrophils, lymphocytes and plasma cells were seen.

Considering the frequency of radioactive nuclei among proliferating cell populations seen in radioautographs of adult mice given single[7] or multiple[8] injections of tritiated thymidine, the mast-cell population sampled in this work was obviously very stable in terms of cell turn-over. If the multiple injections labelled all cells dividing during the 24-hr. period, then it would take 9–18 months to replace completely a population in which only three cells out of 727 were found labelled after the 24-hr. injection period. The three radioactive mast cells listed in Table 1 may represent transformations rather than DNA synthesis by mature mast cells. Radioactive cells which might serve as mast cell precursors were abundant in the areas where mast cells were found (Table 1). Yet, formation of mast cells could not have been the principal objective of mitotic activity in any of these cell populations (including their stem cells) since the number of radioactive fibrocytes, macrophages, lymphocytes and plasma cells was greatly in excess of the number of radioactive mast cells. The frequency of radioactive leucocytes varied with time as would be expected[9], and decreased greatly at the later time intervals.

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Detection of Inclusion Bodies containing Deoxyribonucleic Acid by Means of Autoradiography

We have been examining extensively the *in vitro* multiplication of oil-induced, rabbit peritoneal histiocytes by means of autoradiography. The cells were incubated on flying coverslips in Leighton-tubes, containing 40 per cent homologous serum in Tyrode solution in the presence of 1 μc. tritiated thymidine/ml. In general, little incorporation occurred within the first 48 hr.; but then an increasing number of cells became labelled.

In our first experiments all the label was localized in the nucleus, the cytoplasm remaining free (even in autoradiographs exposed for 6 months). However, in several rabbits which were bought from the same breeder we noticed the presence of a cytoplasmic inclusion body, a finding later closely paralleled in time with the occurrence of a non-bacterial peritonitis. The shape of the inclusion body was that of a star (Fig. 1), and it was quite regular.

This cytoplasmic label appeared irrespective of that of the nucleus. The percentage of histiocytes containing these 'inclusion bodies' increased with time in cell culture. The number of cells containing 2 or 3 bodies in the same cell was also increased.

Table 1 presents quite typical data where several hundred cells were scored for each time period.

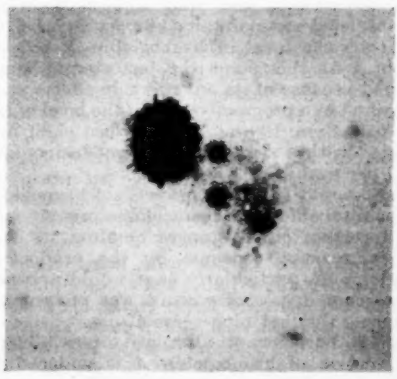

Fig. 1. Cytoplasmic inclusion bodies labelled with tritiated thymidine in rabbit histiocyte

Table 1. Increase with Age of Tritiated Cytoplasmic Inclusion Bodies in Rabbit Histiocytes

Time (hr.)	Percentage of histiocytes labelled
3	0
24	0
48	4
72	9
96	12

It is suggested that autoradiography could be a useful and sensitive method for detection of cytoplasmic parasites containing deoxyribonucleic acid which are in the latent stage but in which turn-over of deoxyribonucleic acid is occurring.

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HÆMATOLOGY

Fœtal Hæmoglobin in Fanconi Type Anæmia

The persistence of fœtal hæmoglobin formation after infancy is usually taken to indicate an anæmic state existing from early life and is most commonly found in association with hereditary hæmoglobinopathies. Despite an extensive search among other types of anæmia only very slight increases have been found, with the exception of quite large amounts present in acute leukæmia in children[1] and in adolescents[2].

A further example of an anæmia developing long after infancy which is also associated with an increased amount of hæmoglobin *F* has been found in a 9 yr. old boy suffering from Fanconi-type anæmia. This child had been under observation from the age of six months because of his poor physical development and the fact that he had congenital deformities of both thumbs. Before the age of 9 yr. the hæmoglobin was estimated to be 10·4–11 gm./100 ml. on three occasions, but at this age a severe anæmia developed with a hæmoglobin of 5·3 gm./100 ml. which was associated with a fœtal hæmoglobin-level of 14 per cent. The hæmoglobin-level was raised by transfusions and, as a result of treatment with methyl testosterone and prednisone as described in ref. 3, this level was maintained for six months, during which time the level of hæmoglobin *F* fell to 6·4 per cent. At this point the testosterone was discontinued because of its adverse side-effects and this has resulted in a progressive fall in the level of hæmoglobin to 7·4 gm./100 ml. over the last eight months associated with a rise in the level of hæmoglobin *F* to 10·7 per cent.

This apparent variation in levels of hæmoglobin *F* with the total amount of hæmoglobin present suggests that in certain circumstances children, as well as neonates, react to anæmia by the production of hæmoglobin *F*, particularly as it would appear that no significant degree of anæmia was present in this case during the first nine years of life.

There is, however, an alternative possibility that, as production of hæmoglobin *A* is under different genetic control from hæmoglobin *F*, its presence here is due to a genetic abnormality associated with the other genetic abnormalities occurring in this condition. It is interesting to note in regard to the earlier finding of hæmoglobin *F* in acute leukæmias that there are on record three instances of Fanconi's anæmia and leukæmia occurring among different members of the same family[4], a remarkable association in view of the rarity of Fanconi's anæmia.

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Agglutination and the Electrical Surface Potential of Red Blood Cells

The reduction of the electrical surface potential of erythrocytes has been considered for a long time as a possible cause of cell agglutination.

Certain colloids coagulate only after decrease of their surface potential below a certain critical value. The same was found for bacteria, the cause of both clumping and electrical discharge being either antibody or acidification[1,2]. Similarly, erythrocytes have been observed repeatedly to show a reduced surface potential after treatment with antibody or with other agglutinating agents.

This communication reports measurements of the surface potential of erythrocytes after treatment with one of the following agglutinating substances: isoantibodies, immune antibodies, agglomerins[3] (specific proteins in plasma of blood with high sedimentation-rate), certain salts and hæmagglutinating viruses. The effects of non-agglutinating (incomplete) antibodies on the surface potential have also been investigated.

A modified Abramson cell electrophoresis apparatus which was adapted for small volumes of test solution (2·0 ml.) was used. This modification permits investigation of suspensions in either salt solutions or protein solutions.

After applying a current of 10·0 m.amp., the velocities of single cells were observed with the microscope in one of the stationary planes of the electrophoresis cuvette and measured by means of a stop-watch and an ocular micrometer calibrated in microns. 10 determinations were made of each sample and the arithmetic mean calculated. The velocities were corrected for field strength (V./cm.) and viscosity. The mobility values were taken as a measure of the electrical surface potential of the cells.

The buffer for diluting most agglutinating materials was a 0·9 per cent sodium chloride solution which contains 5 volumes per cent of *M*/15 phosphate buffer. The final *p*H was 7·2. The (anodic) mobility of human red blood cells in this buffer was 1·080 ± 0·008 μ/sec./V./cm.

All measurements were carried out directly in the appropriate test solutions, adequate time and temperature for incubation being allowed. By this method, the effect on agglutination and surface potential of the medium itself was taken into account. The erythrocyte concentration was 0·1 per cent.

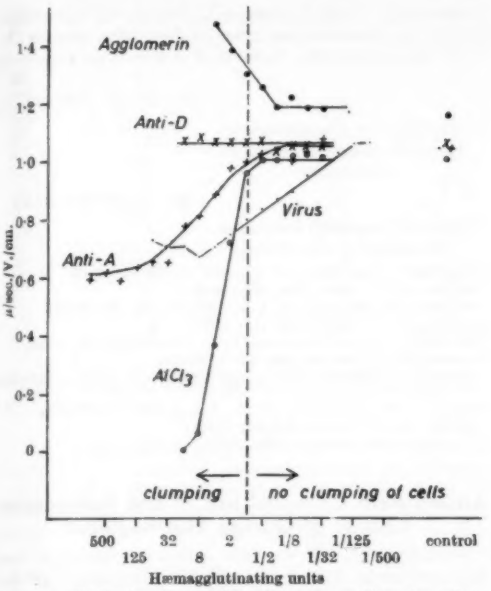

Fig. 1. Electrophoretic mobility of human erythrocytes after treatment with agglutinating agents. Dilutions in geometric progression in phosphate-buffered saline (anti-*A*, anti-*D*, virus), non-buffered saline (aluminium chloride) or compatible normal plasma (agglomeration of blood with high sedimentation-rate)

Following the measurements, the suspensions were recovered and tested for agglutination after 2 min. of centrifugation at 150*g*. In addition, the specific electrical resistance and the viscosity of the supernatant liquids were determined. All measurements including cell electrophoresis were carried out at 23·0° C. Dilutions of all materials in geometric progression in standard buffer were prepared. The highest dilution which gave a macroscopically detectable agglutination was taken as the titre of the solution and was assigned a value of one hæmagglutinating unit.

Typical results are shown in Fig. 1, which indicates that anti-*A* isoagglutinin caused a marked decrease of the electrophoretic velocity of the erythrocytes. However, the effect was small at the concentration at which agglutination began. This behaviour differed from that of hæmagglutinating virus (fowl plague, inactivated by sodium periodate), where a much higher reduction of cell mobility still did not alter the stability in suspension of the blood cells. Anti-*D* had no effect and the electrophoretic mobility remained unchanged even after addition of 32 hæmagglutinating units. Complete and incomplete anti-*D* were electrophoretically similar. The agglomeration of the red blood cells by plasma from a patient with a high sedimentation-rate was accompanied by increase in electrophoretic mobility of the cells. In this case the dilution was carried out with compatible normal plasma. A hæmagglutinating salt (such as aluminium chloride) had a profound effect on cell mobility. No electrophoretic effects were observed on erythrocytes with compatible blood groups. The results will be published later in greater detail.

In summary, the present work indicates several possible relationships between agglutination and surface charge: (1) both agglutination and reduction of the electrophoretic mobility of erythrocytes (like virus); (2) agglutination and no reduction (anti-*D* in saline acting); (3) no agglutination in saline but reduction (special anti-erythrocyte-rabbit sera); (4) no agglutination in saline and no reduction (anti-*D* in albumin acting); (5) increase of mobility in combination with cell agglomeration (plasma of high sedimentation-rate).

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Serum β-Globulin Types in Red Deer and Other Species and their Stability in the Presence of Bacteria

Individual variations in the serum β-globulin patterns obtained on starch-gel electrophoresis have been shown to be genetically determined in a number of species[1–5] and in some the proteins concerned have been shown to be transferrins[7,8]. These variations have been ascribed to a number of alleles[5,6] which have been used in the genetic characterization of animal populations[2,3,7]. This communication reports the serum β-globulin patterns found in red deer (*Cervus elaphus*) with some observations and experiments on their stability, shows that they are due to transferrins and makes some comparisons with the β-globulin patterns in other species.

Serum samples of *C. elaphus* were obtained through several culling seasons from around Braemar, by courtesy of the Mar Estate, and from Rhum, a national nature reserve. A few samples of fallow deer (*Dama dama*) and Sika deer (*C. nipon nipon*) were obtained from parks in Kent. The blood was collected at death and the serum separated before posting to the Rowett Research Institute, where it was stored at + 1° C. for examination. Starch-gel electrophoresis was carried out in phosphate buffer *p*H 7·6 (ref. 9) using hydrolysed starch (Connaught) and paper electrophoresis in 0·05 *M* barbiturate buffer *p*H 8·6 (ref. 5). Transferrins were detected by radioautographs[7] of both starch gel and paper electropherograms of sera to which iron-59 had been added.

The β-globulin zones in *C. elaphus* were found to occupy five possible positions showing a regular decrease in mobility, on starch-gel electrophoresis. Calling these zones *a*, *b*, *c*, *d* and *e*, 254 samples showed patterns of types *ab*, *abc* or *bc*. Six samples showed slower-moving zones of type *cd* or *cde* and two samples showed atypical patterns in which three zones occurred in the region of zones *ab*. Twelve samples of *Dama dama* and five of *C. nipon nipon* were all type *bc*: the zone *b* corresponded to the single β-globulin zone (transferrin) of a human serum. Some examples of the different types of sera are shown schematically in Table 1 and their distribution in *C. elaphus* is given in Table 2.

The samples of *C. elaphus* for the first two seasons were re-examined after storage for several months at + 1° C., when it was found that seventeen of them had changed their type, showing slower-moving zones; this change was also detectable on paper electrophoresis. As an example, five samples of type *ab* become type *cd*. In the subsequent season, care was taken to use a preservative ('Thiomersal', British Drug Houses, Ltd.) in the sample bottles and no

Table 1

Serum β-globulin types, *C. elaphus*, common types, 1 and 2, slow-moving type, 3, atypical pattern, 4. Pattern produced from serum type *ab* 5, *Dama dama* and *C. nipon nipon* also 2. Human, normal sample for comparison 6, pattern produced from same sample 7.

Table 2. Distribution of Serum β-Globulin Types in *C. elaphus*

Zones	*ab*	*abc*	*bc*	Slow	Others	Total
	Samples from Braemar					
Seasons 1958–61	33	14	2	—	—	49
	Samples from Rhum					
Seasons 1958–60	41	36	8	6	1	92
Season 1960–1 (stags)	35	23	2	—	—	60
(hinds)	32	25	3	—	1	61
All seasons	108	84	13	6	2	213

slow-moving types were found. Comparison of the two lots of sera showed that the stored samples had often supported a considerable growth of Gram-positive and negative streptococci and bacilli, accompanied by a slightly more acid reaction.

An attempt was made to produce changes in the β-globulin patterns experimentally by inoculating fresh sera with stored sera from the previous season. Incubation for 24 hr. at 37° C. produced bacterial growth but no changes in pattern; however, on subsequent standing at + 1° C. for four weeks, slower-moving zones appeared and this did not occur in uninoculated control sera. Examples of the changes that can be produced in *C. elaphus* and in human sera are included in Table 1.

Experiments with iron-59 were carried out on various sera to see if the β-globulin patterns being studied were due to transferrins. The radioautographs showed that the zones *a*, *b* and *c* in *C. elaphus* and *b* and *c* in *Dama dama* and in *C. nipon nipon* and the slow-moving zones *c*, *d*, and *e* in *C. elaphus* samples altered on storage were the only ones in the sera combining with iron. This was also found to be true of the strong zones characterizing eight sheep, six cattle and three goat-type sera that have been described[2–4]. The radioautographs of the paper electropherograms confirmed that the proteins studied were β-globulins in all the species mentioned.

It would seem that the various β-globulin types that have been reported are due to transferrins and that these proteins can readily show a stepwise decrease in mobility, comparable to the differences observed between individual types and without affecting the ability of the protein to combine with iron. As the decreased mobility is detectable on paper electrophoresis, the changes are probably due to a decreased negative charge rather than to a gel filtration effect on a system of polymers. A possible explanation of these changes is suggested by the report of Schultze[10] that the bacterial enzyme neuraminidase reduced the electrophoretic mobility of a number of human serum proteins. It would appear advisable, therefore, to accept with caution, as genetic types, rare slow-moving zones, in samples obtained under primitive conditions, unless such changes can be excluded and the type confirmed by a fresh sample from the same individual.

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Amino-Acid Composition of the Polypeptide Chains of Gorilla Hæmoglobin

Comparison of tryptic peptide patterns has demonstrated the great overall similarity of the primary structure of hæmoglobins from normal adult man and anthropoid apes[1]. Work is now in progress to define this similarity more precisely. We report here the determination of the amino-acid composition of the polypeptide chains of gorilla hæmoglobin.

Recent investigation in these laboratories has shown that gorilla hæmoglobin contains two pairs of polypeptide chains. These have the same *N*-terminal sequence by the dinitrophenyl method as the polypeptide chains of human hæmoglobin[2,3]. We designate them, therefore, the α- and β-chains.

The α- and β-chains of gorilla hæmoglobin were separated by chromatography according to the method of Wilson and Smith[4]. Individual chains were hydrolysed in 6 *N* hydrochloric acid at 110° C. for 22 and 70 hr. The amino-acid composition was determined by means of a Spinco automatic amino-acid analyser.

The results are given in Table 1.

On the basis of the amino-acid composition, the α- and β-chains of gorilla hæmoglobin are almost indistinguishable from the corresponding chains of human hæmoglobin. The α-chain of gorilla hæmoglobin appears to have one more residue of aspartic acid and one less residue each of glutamic acid and serine. Amino-acid analysis of tryptic peptides from the α-chain has revealed the substitution of an aspartyl for a glutamyl residue in a peptide equivalent to spot No. 23 (ref. 8) or peptide α*T*-4 (ref. 9) of the α-chain of human hæmoglobin. Assuming an essentially identical sequence in the two types of α-chains, this substitution probably is in the 23rd residue from the *N*-terminus. Whether the α-chain of gorilla hæmoglobin actually contains one less seryl residue is uncertain because of the destruction of serine during hydrolysis. The value given, however, was obtained from each of two independent determinations of the destruction of serine as a function of the time of hydrolysis.

The β-chain of gorilla hæmoglobin clearly contains one less arginyl residue than that of human hæmoglobin and may contain an additional lysyl residue.

Table 1. NUMBER OF RESIDUES PER α- AND β-CHAIN OF GORILLA AND HUMAN HÆMOGLOBINS

Amino-acid	α-chain		β-chain	
	Human*	Gorilla	Human*	Gorilla
ala	21	21	15	15
arg	3	3	3	2
asp	12	13	13	13
cys/2	1	1	2	2
glu	5	4	11	11
gly	7	7	13	13
his	10	10	9	9
ileu	0	0	0	0
leu	18	18	18	18
lys	11	11	11	11 or 12
met	2	2	1	1
phe	7	7	8	8
pro	7	7	7	7
ser	11	10	5	5
thr	9	9	7	7
try	1	1†	2	2†
tyr	3	3	3	3
val	13	13	18	18
Total	141	140	146	145 or 146

* Based on refs. 5 and 6 for the α-chain, on ref. 7 for the β-chain, and on a personal communication from G. Braunitzer.
† Estimated from the tryptic peptide pattern and from the ultraviolet spectrum.

In the hydrolysates of the β-chain, homocitrulline was found. Presumably, this was formed by reaction of lysine with cyanate[10] in the urea which was used in the separation of the chains although its formation would not have been anticipated because of the acidity of the solutions. The value for lysine in the β-chain thus is reported as the sum of lysine and homocitrulline.

These amino-acid compositions thus yield further evidence of the similarity of gorilla and human hæmoglobins. The substitution of an aspartyl for a glutamyl residue in the α-chain and the possible substitution of a lysyl for an arginyl residue in the β-chain are compensatory in charge in each chain. As a result, the electrophoretic behaviours should be alike. Indeed, migration-rates of the two hæmoglobins on paper electrophoresis at *p*H 8·6 are indistinguishable. (Paper electrophoretic migrations were kindly determined by Dr. W. R. Bergren.) The chromatographic behaviours on 'Amberlite *IRC*-50' are detectably, but very slightly, different[11]. Whether the above are the sole differences in composition is, of course, to some extent uncertain because of the usual inaccuracies of amino-acid analysis; for this reason, one cannot be sure that the serine content of the α-chains is different. The amino-acid composition in no way can provide information about changes in sequence without changes in composition.

While these restrictions must be kept in mind, the α-chains of gorilla and human hæmoglobin may well differ by only two residues and the β-chains by one residue. The differences, then, appear to be of the order observed between normal and abnormal human hæmoglobins. Indeed, abnormal human hæmoglobins such as hæmoglobin *S* or hæmoglobin *I* differ from normal human hæmoglobin apparently only by one amino-acid substitution in one or the other chain[12,13]. One might say that the β-chain especially has the characteristics of an abnormal human β-chain although a human β-chain corresponding to the gorilla β-chain has not yet been found. The results suggest that the hæmoglobin genes of man and gorilla constitute a single population.

We wish to express our appreciation of valuable discussions with Prof. Linus Pauling. Mrs. Joan Balog Shelton and Mr. Johnson Cua have given competent technical assistance.

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IMMUNOLOGY

A 'Microspot' Test for Antigens and Antibodies

THE immunologist is often confronted with the problem of determining the presence of low concentrations of antigens in solution, or of establishing the antigenic specificity of a substance available only in minute quantities. On the contrary, he is also frequently concerned with the conservation of small quantities of hard-won antisera. One answer to both these problems is a technique worked out in these Laboratories.

The principle of the technique encompasses the coating of microscope cover-glasses with a thin layer of antiserum-agar, on which microspots of the solutions of the antigens are deposited from fine capillary tubes. The cover-glasses are kept in a moist atmosphere for a suitable period of time and then examined for the presence of spots of antigen-antibody precipitate at the sites of antigen application. In a strong antigen–antibody system precipitation spots will be visible in 1–2 hr. After 24 hr. most positive reactions will be detectable; but it is advisable to keep the cover-glasses for 3 days for the possible appearance of further very weak reactions.

Reading of the moist preparation will generally suffice, and photographs may be made for record. In addition, the preparations may be soaked for periods successively in saline and distilled water and dried at 37° C. The agar film adheres firmly to the cover-glass and a permanent mount is produced. At this stage viewing the surface of the agar by reflected light against a black background may reveal faint spots not apparent in the moist state. The precipitation spots on the dried preparation are readily stained with a suitable dye, such as ponceau *S*, with virtually no background staining. Such stained preparations can be mounted between lantern-slide cover-glasses for projection.

In practice, we have used 5–15 per cent serum-agar for the coating of the cover-glasses, that is, 0·025–0·075 ml. antiserum plus 0·475–0·425 ml.

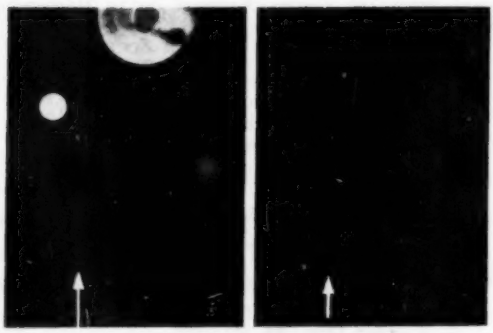

Fig. 1. Comparison of microsp detection of duck ovalbumin in wet preparation (left) and after drying and staining (right). Antigen concentrations, from top: 10,000, 1,000, 100, 10 μgm./ml. 1 per cent specific antiserum in agar gel. (× *c.* 3·5)

1 per cent agar respectively, mixing the two at about 57° C. It is advantageous to use specially purified agar[1]. One-half ml. of agar will readily coat 5–6 one-in. circular cover-glasses, which may be stored in a moist atmosphere until required. No. 3 thickness cover-glasses are preferred for their lower fragility.

The amount of antigen which can be detected depends on the antigen–antibody system involved. We have succeeded in detecting as little as 10 μgm./ml. of duck ovalbumin and the γ-globulin in a 1 : 100 dilution of human serum. We have not yet determined the minimum amount of antibody required in the antiserum to produce a positive test.

We have already applied the technique to the detection of antigen in eluate fractions from chromatographic columns. This, together with the detailed methodology of the technique and alternative use of serum-impregnated papers and membranes, will be reported elsewhere.

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Acrolein and Mengo-Semliki Virus Immunity

A previous report[1] and unpublished qualitative immunochemical findings indicated that acrolein combines with various serum proteins without altering species characteristics. During this work the antiseptic qualities of acrolein were evident by the absence of growth of microscopic organisms; no growth was demonstrable at any time in any of the sealed acroleinized preparations that had been stored at room temperature during a ten-year period. This is of special interest since strict sterile precautions were not exercised during collection of any of the animal sera. These observations, and the known toxic effects of acrolein, suggested application in the preparation of vaccines.

This initial report concerns animal experimental work with acroleinized Mengo and Semliki viruses. Mengo and Semliki Forest viruses were chosen for this initial acute experiment because of their known lethal virulence for mice. Paralysis and death following infection occur in unprotected mice over a period of 1–8 days, depending on the age of the mice, route of administration and the number of lethal doses of live virus injected.

Stock virus was obtained from the Department of Clinical Virology, Sloan-Kettering Institute: Mengo virus: stock 4, dated November 10, 1958 (unknown number of brain passages), stored frozen as 20 per cent mouse brain supernatant with 5 per cent human serum-saline; intracranial titre, 10^{-8} in 5–6-week-old mice; Semliki Forest virus: stock 4, dated December 3, 1959 (8th brain passage), stored frozen as 20 per cent mouse brain supernatant in 5 per cent mouse serum-saline; intracranial titre, 10^{-7} in 5–6-week-old mice. Swiss Webster mice, approximately 5 weeks old, weight 16–18 gm., were used. Acrolein (bulk acrolein stabilized with hydroquinone; maximum hydroquinone 0·1–0·2 per cent, supplied by Dr. T. J. Hall, Union Carbide Chemical Co., New York, N.Y.): 0·2 per cent in 0·85 per cent chilled sterile saline was freshly distilled and used immediately in each experiment.

Stock Mengo virus (20 per cent) was diluted tenfold with sterile distilled water to 2×10^{-3}. Stock Semliki virus (20 per cent) was diluted tenfold to 2×10^{-3}. This was injected intracranially in two groups of mice in 0·3-ml. doses per mouse. Brains from only the living moribund as well as the clinically well Mengo- and Semliki-infected mice were collected rapidly and aseptically and pooled approximately 24 hr. and 44 hr. respectively following injection. This was not titred. The brain tissue was immediately macerated with scissors and homogenized in a 'Tri-*R* Teflon' tissue homogenizer with a small quantity of 0·2 per cent acrolein necessary to maintain a liquid state. Following homogenization, sufficient chilled 0·2 per cent acrolein was added to make a final 20 per cent brain suspension. The suspensions were put in brown sterile rubber-stoppered vaccine bottles, exposed to room temperature for 24 hr. and stored in a refrigerator at 1–5° C. As control for the detection of live virus, Mengo and Semliki vaccines 12 days and 28 hr. respectively after preparation were serially diluted tenfold with sterile distilled water and injected intracerebrally in 0·03-ml. doses in groups of mice approximately five weeks old. There was no evidence of illness or death over an observation period of 15 days, at which time the animals were killed.

In a similar test experiment, live stock Mengo virus, diluted 1 : 10 with 0·2 per cent acrolein, was shown to be inactivated after exposure for 30 min. at room temperature. (Incidentally, 0·2 per cent acrolein inactivates Rous sarcoma, Friend's leukæmia and Egypt 101 viruses after 24 hr. exposure at room temperature.) Tissue cultures for the detection of live virus were not carried out.

For immunization tests, mice of the same delivery tag-date age were divided into three separate groups. One group was vaccinated with Mengo vaccine (age, 3 days) subcutaneously; one group peritoneally; and the third group was unvaccinated to be used as controls. Vaccination consisted of a 0·1-ml. injection of acroleinized 20 per cent suspension of mouse brain initially, repeated every seven days for a total of four injections. Ten days following the last injection, the vaccinated and unvaccinated controls were challenged intraperitoneally with live stock Mengo virus. With the Semliki vaccine (age, 1 day), mice of the same tag-date age were divided into four groups. Two groups were vaccinated intraperitoneally, receiving 0·1 ml. of vaccine initially, repeated every seven days for a

Table 1. MORTALITY FOLLOWING INOCULATION WITH LIVE VIRUS (DEAD/TOTAL)

Live virus inoculated	Group and route of challenge	2×10^{-1}	Virus dilution 10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}
Mengo	Unvaccinated controls (*IP*)		5/5	5/5	5/5	5/5	5/5	4/5	1/5
	Intraperitoneal vaccinated (*IP*)*		0/5	0/5	0/5	0/5	0/5	0/5	0/5
	Subcutaneous vaccinated (*IP*)		1/3	1/3	1/3	1/3	1/3	1/3	0/3
Semliki	Unvaccinated controls (*IC*)	3/3	5/5	5/5	2/4				
	Intraperitoneal vaccinated (*IC*)*	0/3	0/5	0/6	0/3				
	Unvaccinated controls (*IP*)		3/5	1/5	0/5	0/5	0/5	0/5	0/5
	Intraperitoneal vaccinated (*IP*)*		0/5	0/5	0/5	0/5	0/5	0/5	0/5

* No deaths over a 15-day period of observation.

Intraperitoneal (*IP*), intracranial (*IC*) route of administration of live virus.

total of four injections. Two groups remained unvaccinated for use as controls. Ten days following the last injection, the four groups were challenged with live stock Semliki Forest virus.

Results are summarized in Table 1. (1) The group vaccinated intraperitoneally was protected against approximately one million (LD_{50}) lethal doses of live Mengo virus, while the group vaccinated subcutaneously showed only irregular partial protection. (2) The vaccinated group challenged intracerebrally was protected against approximately 2,000 (LD_{50}) lethal doses of Semliki Forest virus. It is interesting to note that three mice from each of these groups received undiluted 20 per cent stock virus (2×10^{-1}). The results of challenge via the intraperitoneal route in the other two groups shows a one log protection in the vaccinated mice.

Space does not permit a detailed discussion; however, it appears that acrolein stabilizes without altering the basic compound molecular pattern of the viruses studied. While the molecular configuration was probably altered to some extent by acrolein, there was no noticeable loss in the capacity of the acroleinized virus to act as an antigen when injected into mice. The immune response was determined by the ability of the vaccinated mice to survive challenge against overwhelming lethal doses of live virus injected, and not by the test-tube type determination, based on the amount of reactive humoral substances present in serum.

I am indebted to Dr. Emerson Day for his co-operation and help in initiating this work and to Dr. Chester Southam for his criticism and giving me facilities for this investigation.

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PATHOLOGY

Absorption and Excretion of Penicillin injected into the Muscles of Patients with Diabetes Mellitus

THE observation that two patients with sub-acute bacterial endocarditis due to alpha-streptococci highly sensitive to penicillin *in vitro* failed to respond to administration of six million units of the drug a day led to investigations which revealed low peak blood-levels and abnormal urinary excretion of the antibiotic in both. When the dose of penicillin was increased to a quantity greatly in excess of that suggested by the test-tube sensitivity of the organisms, favourable clinical response followed promptly. Other than the endocarditis, the only finding common to both individuals was the presence of diabetes mellitus. Since a review of published information concerning the pharmacology of penicillin[1-4] revealed no data indicating any abnormalities related to the diabetic state, it was decided to investigate this problem.

Two types of subject were investigated: (*a*) patients with established diabetes mellitus controlled by diet alone or with insulin; (*b*) individuals with no clinical or laboratory evidence of diabetes who were approximately the same age as the diabetics. They were selected on this basis in order to eliminate, so far as possible, the effect of age on the efficiency of renal tubular excretion of penicillin. A single dose of 10,000 units (6 mgm.) of crystalline benzyl penicillin *G* per kgm. of body-weight was injected into the gluteus. Blood and urine specimens were obtained before treatment and 1/2, 1, 2, 4, 6, 8, 24, and 48 hr. after antibiotic administration. Concentration of penicillin in the blood was determined by the tube-dilution and agar-cup methods. The quantity of drug in the urine was estimated by the tube-dilution technique. The test organism used was *Staph. aureus* (var. *pyogenes*) *FDA* 209*P*.

Thirty-one diabetic subjects and twenty-nine normal 'controls' were investigated. There was some variation in the rates of absorption and excretion of penicillin, some individuals with diabetes being indistinguishable in this regard from those without this disease. However, as illustrated in Fig. 1, calculated averages revealed striking differences. Peak blood-levels were reached earlier (1 hr.) and were higher (8 units/ml.) in the controls than in the diabetic patients (2 hr. and 5·4 units/ml. respectively). Disappearance of penicillin from the serum appeared

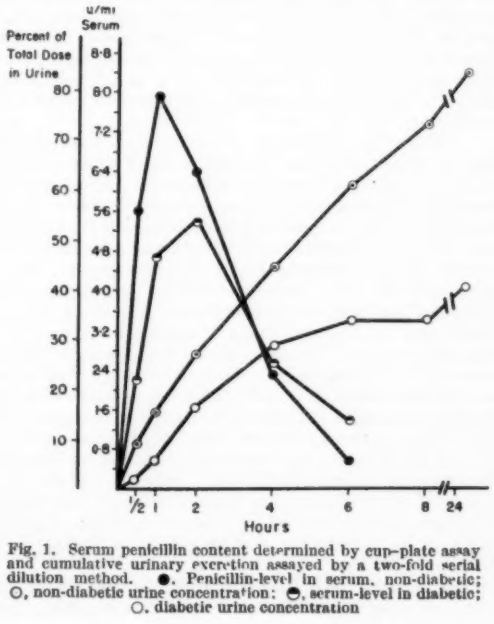

Fig. 1. Serum penicillin content determined by cup-plate assay and cumulative urinary excretion assayed by a two-fold serial dilution method. ●, Penicillin-level in serum, non-diabetic; ○, non-diabetic urine concentration; ◓, serum-level in diabetic; ○, diabetic urine concentration

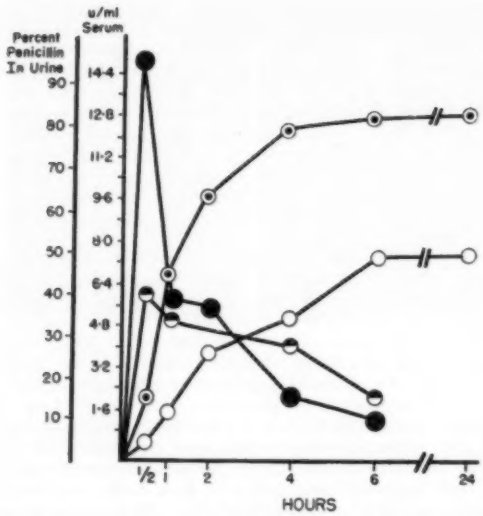

Fig. 2. Penicillin content of serum and urine in one diabetic subject after both intravenous and intramuscular administration. ○, Cumulative penicillin excretion in urine of diabetic after intravenous dose 10,000 units/kgm.; ○, penicillin excretion after intramuscular dose of 10,000 units/kgm.; ●, serum content after intravenous dose; ◒, serum content after intramuscular dose

to proceed at the same rate in both groups. The half-life of the antibiotic was found to be almost identical, 0·79 hr. in the non-diabetics and 0·83 hr. in those with diabetes.

The urinary excretion of penicillin by diabetic patients, although somewhat variable, was, on the average, very different from that of the controls (Fig. 1). The normal subjects cleared approximately 50 per cent of the injected dose of drug via the kidney in 2 hr., 80 per cent after 8 hr., and 95 per cent after 24–48 hr. The average urine output of antibiotic in diabetics was found to be only 25 per cent after 2 hr. and 50 per cent after 24 hr. Specimens of urine obtained from both groups after 36 hr. contained too little penicillin to be detected by the method used, since concentrations less than 10 units/ml. were not measured.

Since the data obtained indicated that the absorption and excretion of penicillin were abnormal in diabetes mellitus, investigations were carried out to determine the responsible mechanism. The possibility that variation in degree of protein-binding of the antimicrobial agent might be involved was eliminated by failure to demonstrate significant changes in antimicrobial activity when penicillin, in varying quantities, was incubated *in vitro* with serum, plasma, whole blood, and urine of diabetic and normal subjects. The fact that the half-life of the antibiotic was found to be essentially the same in both groups suggested that (*a*) derangements in kidney function associated with the diabetic state, (*b*) destruction of the drug, or (*c*) a metabolic pathway other than that available in normal individuals was probably not responsible for the reduced serum-levels and renal clearance in diabetes. An investigation was undertaken, therefore, to determine the role of the site of injection of penicillin on its absorption and excretion in normal and diabetic patients.

One patient with diabetes mellitus controlled by diet and insulin has thus far been investigated. He was first given the standard dose of penicillin intramuscularly and blood and urine concentrations were determined 1/2, 1, 2, 4, 6, and 24 hr. after treatment. Concentrations of drug in the blood were determined using the agar-cup method and in the urine by the tube-dilution technique. Several days later, he was given the same dose of penicillin intravenously and blood and urine collected at the same intervals. The results of this work are presented in Fig. 2. The curves of serum and urine concentrations when penicillin was given intramuscularly approximate closely those presented in Fig. 1 as an average for thirty-one diabetic subjects. Quite distinct are the blood-levels and cumulative urinary excretion following intravenous administration of the antibiotic; these resemble closely those presented in Fig. 1 as average for twenty-nine 'controls'. The total quantity of penicillin excreted in the urine was considerably larger when it was given intravenously than when it was injected into a muscle. These results suggest that the low peak blood-levels and decreased urinary excretion of antibiotic noted in patients with diabetes mellitus are probably due to defective absorption from an intramuscular depot and not to altered renal function, increased rapidity of destruction, excessive protein binding, or metabolism via an unusual pathway.

The observations which have been presented appear to be significant not only because they indicate that absorption of penicillin from intramuscular sites is abnormal in diabetes mellitus, but also because they suggest the possibility that other substances may also be absorbed relatively poorly and that this represents a basic absorptive defect in this disease.

This work was carried out under a grant from Lilly Laboratories, Indianapolis, Indiana.

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Establishment of Amœbic Hepatitis as a Distinct Clinico-Pathological Entity

There is much controversy as to whether or not amœbic hepatitis occurs as an entity, as distinct from the well-recognized amœbic liver abscess. Recently, we reported a case of long-standing amœbiasis with hepatomegaly which, on liver biopsy, showed histologically the presence of cells morphologically similar to amœbæ, diffusely infiltrating the liver without abscess formation[1].

Further support to our concept of amœbic hepatitis is given by two patients on whom we now report. These patients, both with amœbiasis and hepatomegaly without liver abscess, were found to have cells, morphologically similar to amœbæ, diffusely infiltrating the liver on biopsy examination.

On laparotomy, the liver of both patients had a fine granular appearance and histologically showed inflammatory infiltration and some portal fibrosis. Liver tissue from the biopsies was injected into the

liver of hamsters, guinea pigs and rabbits. In the hamsters and the rabbits, liver abscesses were produced which showed the presence of cells morphologically similar to amœbæ; such cells had also diffusely infiltrated the liver.

The passage of the disease from the human to the experimental animals, we believe, gives further support to the view that diffuse amœbic hepatitis exists as a distinct clinico-pathological entity.

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ANATOMY

Third Molar Polymorphism and the Timing of Tooth Formation

AGENESIS (congenital absence) of the third molar tooth is a familiar polymorphism in man, with a population frequency of at least 9 per cent in Europeans[1]. Hitherto, congenital absence of this tooth has been considered as an independent trait, or at most associated with reduction in size when agenesis is incomplete. However, there is evidence connecting the presence or absence of the third molar tooth with the timing of formation of the premolar and molar teeth as well.

In a series of 172 white children born in Ohio (excluding monozygotic pairs), agenesis of the lower left third molar was radiographically confirmed in 22 subjects aged fourteen years or older. The time of formation of the remaining lower left premolar and molar teeth was determined from serial, longitudinal oblique-jaw radiographs[2] and expressed as sex-specific normalized T-scores[3] so that data on boys and girls could be combined. As shown in Table 1, children lacking the mandibular left third molar were consistently later in premolar and molar tooth formation than 126 'controls' from unaffected sibships.

Table 1. CALCIFICATION TIMING OF THE POSTERIOR TEETH IN CHILDREN LACKING $\overline{M}_3$, THEIR SIBLINGS, AND IN CHILDREN FROM UNAFFECTED SIBSHIPS

Group	Calcification timing									
	$\overline{P}_2$		$\overline{P}_2$		$\overline{M}_1$		$\overline{M}_2$		$\overline{M}_3$	
	N	T^*	N	T^*	N	T^*	N	T^*	N	T^*
Affected children	21	53	20	54	18	52	21	59	—	—
Siblings of above	22	56	20	53	17	52	24	52	24	57
Unaffected children	111	48	118	49	81	48	126	48	125	49

* Normalized sex-specific T-scores (ref. 3) for beginning tooth formation, primarily beginning cusp calcification (ref. 4).

Furthermore, 24 unaffected siblings of children with third molar agenesis were compared with the unaffected children from unaffected sibships. These unaffected siblings of the propositi also proved late in tooth formation, especially for mandibular third molar formation which averaged 8 T-scores behind the controls (Table 1). For all 150 unaffected children, earliness or lateness of $\overline{M}_3$ formation was significantly associated with the type of sibship from which they came ($\chi^2 = 6{\cdot}4$). Thus, the tendency for delayed tooth formation when the third molar is missing is characteristic of affected lineages and not restricted to affected individuals alone.

Since absence of the mandibular third molar was associated with delayed formation of the molar teeth, it was not surprising to find differences in the sequence of tooth formation[4] between the 22 children with third molar agenesis and the 126 normal controls. Thus, 60 per cent of children in the present work who lacked the mandibular third molar were of the P_2M_2 sequence of cusp calcification, while this sequence or order was far less common (22 per cent) in the 126 controls. Third molar polymorphism was unquestionably associated with the P_2M_2/M_2P_2 sequence polymorphism ($\chi^2 = 25{\cdot}01$).

Third molar agenesis, therefore, may be viewed as the extreme degree of expression of factors delaying tooth formation over a long developmental period ranging from the first month of life (for M_1) to the eighth year or beyond (for M_3). Moreover, this common polymorphism is related to the previously described familial P_2M_2/M_2P_2 calcification sequence polymorphism[5], indicating a common determinant for the two.

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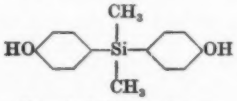
HISTOCHEMISTRY

Allergy to a Carbon-Functional Organic Silicon Compound, Dimethyldi-(4-hydroxyphenyl)-silane

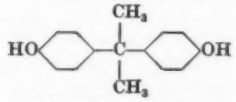
THE noxious effects of certain inorganic silicon compounds are well known[1]. Polymeric organic silicon compounds, on the contrary, are chemically very inert and show a low toxicity[2]. Some organic silicon compounds may, however, produce toxic effects[3].

Inorganic or organic silicon compounds—the latter not present in Nature[4]—are so far not known as allergens.

As a close relationship exists between silicon and carbon atoms, it was assumed that cross-sensitization could occur between silicon and carbon compounds. Therefore the allergenic nature of dimethyldi-(4-hydroxyphenyl)-silane[5] was investigated since the carbon compound, 2,2-*bis*(4-hydroxyphenyl)propane ('Bisphenol *A*'), has been demonstrated as an allergen[6].



dimethyldi-(4-hydroxyphenyl)-silane



2,2-*bis*(4-hydroxyphenyl) propane

Four non-atopic females with hypersensitivity to 2,2-*bis*(4-hydroxyphenyl)propane, demonstrated by positive patch test (1/100 dilution in ethyl alcohol), showed positive reactions to dimethyldi-(4-hydroxyphenyl)-silane (1/100 dilution in acetone). The positive tests were characterized by the presence of erythema, infiltration and papules/vesicles.

This is the first report on allergy to an organic silicon compound. Absence of earlier contact with the silane is evidence of cross-sensitization.

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Use of Tetrazolium Compounds in Oxidative Enzyme Histo- and Cyto-Chemistry

A new tetrazolium salt previously synthesized has been evaluated for its ability to overcome the technical and theoretical barriers of either the MTT-Co (ref. 1) or nitro-BT methods[2,3]. This compound is TNBT or 2,2′,5-5′-tetra-*p*-nitrophenyl-3,3′-(3,3′-dimethoxy-4,4′-biphenylene)-ditetrazolium chloride (Fig. 1), the formazan of which is purplish black[4]. The TNBT used in the following investigations produced a brownish-black precipitate in tissue sections. Though quite low in water solubility, minimal concentrations of TNBT are required for the effective demonstration of the succinic dehydrogenase system in fresh, frozen sections of tissue of mammalian tissues or human vaginal smear preparations. The following were investigated in mouse myocardium and kidney: (1) effect of concentration of dye on the products of TNBT formazan in slices of these tissues; (2) effect of *p*H on the reduction of TNBT by the succinic dehydrogenase system in tissue sections in the presence or absence of appropriate substrate; (3) solubility of TNBT formazan in aqueous, alcoholic and other organic solvents necessary for the effective resin-mounting of sections of tissue containing precipitates of formazan.

Results indicate that :

(1) Varying the concentration of TNBT in the incubating medium from 0·001 mgm./ml. to 1·25 mgm./ml. through the use of an appropriate solvent has considerable effect on activity of the succinic dehydrogenase system in sections of heart and kidney. The best concentrations of this tetrazolium appear to be in the range 1·25 mgm./ml.–0·25 mgm./ml. (Figs. 2 and 3).

(2) Maximal deposition of TNBT formazan occurs in succinic dehydrogenase-stained tissue sections over a wide range of *p*H. Tissues incubated in TNBT solutions containing succinate and phosphate buffer with *p*H values of 6·8–8·4 show heavy staining reactions with little variation in intensity as a result of the concentration of hydrogen ions. In the absence of succinate, extremely pale non-granular tints appeared in the tissue sections, especially at *p*H 8·4. The very faint staining, when visible in such specimens, was distinctly different from the TNBT formazan granulations seen in localizations of the succinic-dehydrogenase system.

(3) TNBT formazan, enzymatically produced in tissue sections or in non-enzymatic systems using dithionite, is as insoluble as nitro-BT formazan in any of the ordinary organic solvents used in the resin-mounting of tissue sections.

(4) Crystallization phenomena such as those observed in nitro-BT formazan specimens (Fig. 4) have never occurred in TNBT-stained tissue sections (Fig. 5).

(5) The granular precipitate of TNBT formazan can be of such a fine dimension, suggesting a more correct staining picture rather than that of an

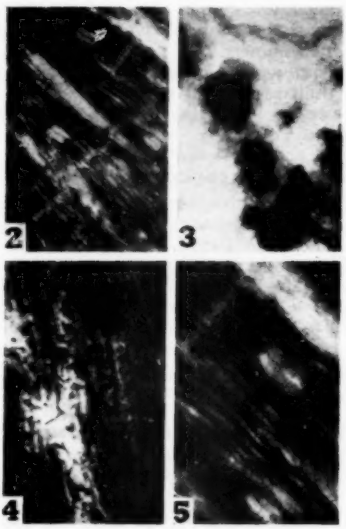

Fig. 2. Mouse myocardium stained for the succinic dehydrogenase system using TNBT. The muscle cells are well stained and cross-striations are evident in many regions of the myofibrils. Note the absence of staining in nuclei and intercalated disks (upper right, lower left, centre). 5-μ section. (× 300)

Fig. 3. Mouse kidney stained for succinic dehydrogenase system using TNBT. Heavy deposition of the diformazan is seen in the proximal tubules while no staining is apparent in the glomerulus (left). Note faint staining of smooth muscle in the tunica media of the artery (top). 10-μ section. (× 60)

Fig. 4. Mouse myocardium stained for the succinic dehydrogenase system nitro-BT. Coarse granular precipitate marks the site of enzyme while occasional crystals can be seen (middle) in extracellular regions. 5-μ section. (× 600)

Fig. 5. Mouse myocardium stained for the succinic dehydrogenase system using TNBT. The fineness of the diformazan precipitate sharply delineates the mitochondria within the muscle cells. In addition, note the clearly defined inactive nucleus (centre) under which is situated a portion of a stained myofibril. Unstained intercalated disk (upper left). 5-μ section. (× 600)

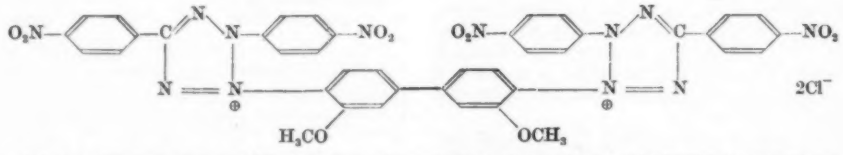

Fig. 1. Structural formula of 2,2′,5,5′-tetra-*p*-nitrophenyl-3,3′-(3,3′-dimethoxy-4,4′-biphenylene)-ditetrazolium chloride (TNBT)

accumulated precipitate over intracellular organelles. This localization is under investigation at present using electron histochemical techniques similar to the procedures of Sedar and Rosa[5].

(6) Cyanide inclusion into incubating media shows little effect in highly active tissues such as heart and kidney. Its use for enhancement of staining in tissues of the female reproductive tract appears to be warranted, especially in regions where low succinic dehydrogenase activity prevails.

In conclusion, TNBT appears to possess qualities suitable for the histo- and cyto-chemical localization of oxidative enzymes in tissue sections of mouse heart and kidney. These properties strongly suggest that TNBT may be a more efficient reagent for the cytochemical demonstration of enzyme systems such as succinic dehydrogenase and diphosphopyridine nucleotide diaphorase.

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BIOLOGY

Anaerobiosis and Sodium Accumulation

Accumulation of most cations by higher plants is considered to be an aerobic process. However, we now find that sodium is in part an exception to this generalization. Uptake of sodium by 6-day old plants of *Hordeum vulgare*, variety Atlas 46, occurs under both aerobic and anaerobic conditions. Accumulation in the roots under anaerobic conditions is restricted to a period of about 2 hr. (Fig. 1), during which time transport to the shoots is almost nil (Fig. 2). The amount of sodium accumulated by plants the roots of which are in either aerobic or anaerobic solutions is inhibited by substances such as iodoacetate and methylene blue, indicative of the initial uptake being coupled to metabolic reactions in both cases rather than to passive processes. Non-steady-state uptake by the anaerobic plants, which decreases with time as if some process is attenuating, suggests that the reactions involving sodium uptake are several steps removed from the respiratory chain.

Previously observed accumulation of sodium in plants under anaerobic conditions[1-3] over long periods of time was often considered in discussion as possible passive entry due to plant damage. That the plants are not damaged by anaerobiosis in the present experiments is indicated by return to normal respiration after 60 min. of anoxia and 15 further hours under aerobic conditions. At the end of this treatment the rate of sodium accumulation in the shoots is increased but can be stopped by returning the plants to an anaerobic situation or by the use of inhibitors. Anaerobiosis *per se* does not activate sodium uptake; rather it increases the rate of sodium uptake on returning to aerobic conditions.

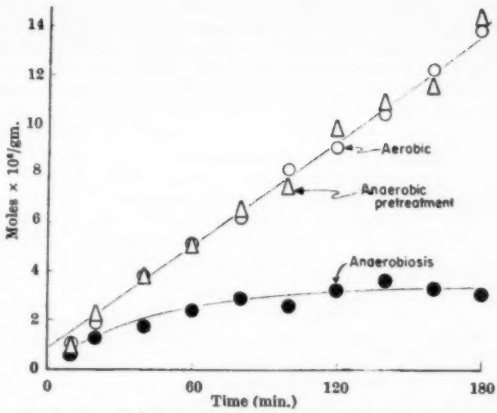

Fig. 1. Accumulation of sodium ions by roots from a 10^{-3} *M* solution at pH 5·0

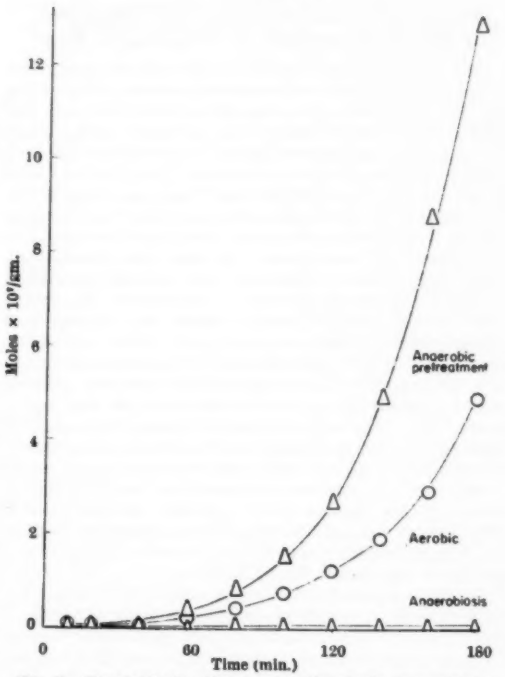

Fig. 2. Transport of sodium ions to the shoots from 10^{-3} *M* solution at pH 5·0

Activation of sodium uptake by shoots produced by anaerobic pretreatment of roots, either by bubbling nitrogen or carbon monoxide gas through the solution, occurs chiefly from the higher sodium concentrations ($> 10^{-4}$ *M*). Two or more reactions appear to be involved in the uptake of sodium by roots[4] and shoots. The activation of anaerobic pretreatment is on the reaction for shoot accumulation which dominates at the higher sodium concentrations.

The uptake of potassium and phosphate differs from sodium accumulation in respect to anaerobiosis.

Both potassium and phosphate accumulation are immediately suppressed by anaerobic conditions and return only to the normal value in air (no enhancement). Accumulation was followed in all cases by the amount of radioisotopes appearing in the roots or tops.

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Effect of Environment on the Responses of Dwarf Groundsel to Gibberellic Acid

THE striking effects of gibberellic acid on internodal elongation in dwarf mutants and rosette plants of a number of species are now well established, and the effects of this substance on a single gene dwarf mutant, induced by γ-radiation in groundsel, *Senecio vulgaris* L., have recently been described[1]. The observations now reported show that the responses of this mutant to gibberellic acid may be considerably affected, both quantitatively and qualitatively, by environmental conditions. In fact, the phenotypic expression of both wild-type and mutant groundsel is greatly influenced by the environment: in certain unfavourable conditions the plants are stunted and the vegetative phase is prolonged. When the wild-type (tall) and mutant (dwarf) groundsel are grown in a greenhouse during the normal growing season, that is, from early spring to late summer, the transformation of the vegetative apex to an inflorescence apex occurs not later than 2 weeks after the emergence of seedlings from the soil, and fruiting begins when plants are 7–10 weeks old; but the apices of tall and dwarf plants grown in a growth cabinet under 'daylight' fluorescent tubes, 400 foot-candles, with a day-length of 10 hr., and a temperature of 22 ± 2° C., remained vegetative for 11 weeks.

Dwarf groundsel has shorter and fewer internodes than the wild-type plants of the strain from which it was derived. Mitotic activity is significantly less in the apical meristem and subapical region of the shoot in dwarfs than in wild-type plants. Treatment with gibberellic acid of dwarfs grown in a greenhouse leads to a conspicuous and precocious extension of the internodes due to enhanced mitotic activity and cell elongation in the subapical region of the shoot[1]. No evidence of any response of the tissues of the apical meristem was obtained, and there was no significant increase in the number of nodes.

Both tall and dwarf genotypes in the growth cabinet grew poorly and remained stunted. In these conditions, however, the genetic dwarfs responded proportionately more strongly to gibberellic acid than when they were grown in the greenhouse. Treated mutants were taller than untreated tall plants of the same age in the same environment, although they were not even as tall as untreated dwarfs grown in the greenhouse. An increase in the number of leaves, and thus of internodes, on the main axis of treated dwarfs in the growth cabinet was also observed (Table 1), and since leaf primordia originate on the apex, such an increase must reflect the influence of gibberellic acid on the apical meristem.

Table 1. EFFECT OF ENVIRONMENT ON THE HEIGHT AND NUMBER OF INTERNODES OF TALL AND DWARF GROUNDSEL AND THOSE TREATED WITH GIBBERELLIC ACID (GA)

Type of plant	Mean final height (cm.) Exp. *A*	Exp. *B*	Mean number of internodes Exp. *A*	Exp. *B*
Tall, control	40·4 (1·05)	2·2 (0·27)	14·2 (0·46)	15·5 (2·40)
Tall, GA-treated	45·2 (1·05)	8·0 (3·44)	13·8 (0·58)	17·6 (0·70)
Dwarf, control	8·6 (1·01)	0·6 (0·16)	7·9 (0·16)	10·8 (1·27)
Dwarf, GA-treated	39·1 (1·52)	5·9 (2·53)	8·5 (0·63)	16·6 (1·56)

95 per cent confidence interval given in parentheses.

Exp. *A*, greenhouse: 18 plants in each group, measured at 47 days old when all plants were fruiting. Treated plants received 10 p.p.m. gibberellic acid at weekly intervals (6 treatments).

Exp. *B*, growth cabinet: 6 plants in each group, measured at 77 days old; most plants were still vegetative at the time of the final measurement, but appeared unlikely to survive to complete the life-cycle. Treated plants received 10 p.p.m. gibberellic acid twice weekly for the first three weeks, and once a week thereafter (13 treatments).

In order to obtain apical material from plants in the growth cabinet comparable with 8-day-old seedlings from the greenhouse, 20-day-old seedlings had to be selected, because of the slower growth and prolonged vegetative phase of plants in the growth cabinet. Observations of longitudinal sections of shoots from the growth cabinet which had been treated with 10 p.p.m. gibberellic acid twice weekly (6 treatments), showed that while the most striking morphogenetic effect of gibberellic acid was again on the sub-apical tissue of treated dwarfs, there was also a significant ($P = 0.05$) effect on the apical meristem. Treatment with gibberellic acid of dwarfs grown in these special conditions enhances cell division in the apical meristem and brings it to the level observed in normal tall plants grown in the same environment (Table 2). Since there were fewer than 10 mitotic figures in the apical and subapical regions of most shoots, tests of significance were carried out on data transformed by the method $\sqrt{X + 0.5}$, where X is the number observed[2]. As already stated, when groundsel seedlings were grown in greenhouse conditions where the vegetative phase is of relatively brief duration, no indication of any morphogenetic response of the apical meristem to gibberellic acid was obtained, and this is in accord with observations on other species[3,4]. When, however, groundsel seedlings were grown in an environment in which the initiation of the reproductive phase was delayed, permitting a longer period of gibberellic acid treatment while the apex was vegetative, there was clear evidence of a response of the apical meristem.

It may well be that stem elongation in normal and dwarf groundsel is modified by two major factors, namely, photosynthesis, which provides the basic substrates for growth, and gibberellin, which regulates the distribution of growth; and to the extent that the latter determines what proportion of the

Table 2. MEAN NUMBER OF MITOSES IN A MEDIAN PANEL 36μ IN THICKNESS (6 MEDIAN LONGITUDINAL SECTIONS, 6μ PER SECTION) OF SHOOT TIPS IN GROUNDSEL

Type of plant	Mean number of mitoses: Apical meristem Exp. *A*	Exp. *B*	Subapical region Exp. *A*	Exp. *B*
Tall (wild-type) plant	6·0	3·8	4·6	3·6
Untreated dwarf	3·2	2·0	1·0	1·3
GA-treated dwarf	3·2	3·6	6·4	6·4

Exp. *A*. Greenhouse: 5 plants, 8 days old, treated with 10 p.p.m. gibberellic acid on alternate days (4 treatments).

Exp. *B*. Growth cabinet: 10 plants, 20 days old, treated with 10 p.p.m. gibberellic acid twice weekly (6 treatments).

products of photosynthesis is incorporated in leaf tissue, changes in it lead to changes in the former.

It seems likely that the heights of dwarf and normal plants grown in the greenhouse were limited, though to markedly different degrees, by deficiency of endogenous gibberellin, since both types of plant responded to applied gibberellic acid, the former strongly, and the latter relatively slightly. The growth of dwarf plants was also limited by the relatively deficient photosynthesis, since the number and size of leaves are characteristically less in dwarfs than in normal plants. Photosynthetic tissue was increased in greenhouse-grown dwarfs by gibberellic acid treatment, due to an increase in the size, but not in the number of leaves. (Photosynthetic efficiency, measured as net assimilation-rate, was similar in normal and dwarf plants, and was not affected by gibberellic acid[1].)

The growth and development of untreated and gibberellic acid-treated dwarf and normal plants grown under low light intensity in the growth cabinet was profoundly modified by reduced photosynthesis; and, in consequence, the morphogenetic response of the dwarf to gibberellic acid was modified, since during the protracted vegetative phase the apical meristem responded to gibberellic acid, and the treated dwarfs produced as many leaves as normal plants. Thus the effect of gibberellic acid on the photosynthetic tissue of the dwarf mutant was relatively greater when the plants were grown in the growth cabinet than when they were grown in the greenhouse, and this may have accounted for the relatively increased stem elongation of treated dwarfs in these conditions. Stem elongation was greater in these treated dwarfs than in untreated normal plants which were probably deficient in natural gibberellin, as indicated by the response of normal plants to treatment with gibberellic acid.

It is evident that the morphogenetic responses of *S. vulgaris* to gibberellic acid are considerably modified by environmental factors, and it seems probable that this is due to effects on both the photosynthetic capacity of the plants and their native growth substances, including gibberellin. It may well be that an investigation of the responses to gibberellic acid of dwarf mutants in other species grown in different environments would be of interest, and might lead, as in this case, to still more striking demonstrations of the effects of this substance.

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Volatile Mustard Oils of *Brassica juncea* Seeds

Brassica juncea Coss. and Czern. is an important cultivated mustard species. It is said to be a native of Asia and is grown particularly in China, Japan, India, Pakistan, Nepal and eastern Europe as a vegetable and an oil-seed crop, although it has also been introduced into North America and certain West European countries as an oil-seed crop and for use in the manufacture of condiments[5]. The species is of botanical interest in that it shows marked variation in external morphology, especially leaf form[1]. Variation, too, has been reported in the type of volatile oil produced on the hydrolysis of the seed glucoside; three conditions have been described: (*a*) the production of allyl isothiocyanate only[2,4]; (*b*) the production of 3-butenyl (= 'crotonyl') isothiocyanate only[4]; (*c*) the production of a mixture of allyl and 3-butenyl isothiocyanates[2].

In present investigations into variation in *B. juncea* in connexion with a programme of plant breeding, 96 samples of seed were obtained from a wide range of geographical sources, and grown in England; this collection was found to include all the main varietal forms of the species previously described in the literature. The volatile mustard oils of the seeds were identified using a paper chromatographical method[3]; in this analysis two controls were run, one of allyl thiourea and the other of phenyl thiourea (which gave a reference spot from which *Rph* values of other compounds were defined). Allyl isothiocyanate gave an *Rph* value of 0·26 and the value of the only other compound detected in the test, 0·62, indicated that it was 3-butenyl isothiocyanate.

All the three conditions of volatile oil production previously described were found during these investigations, and there appears to be a correlation between this variation and the geographical distribution of the species, a situation, so far as is known, not previously reported. Seeds from China, Japan, Nepal and Eastern Europe (49 samples) exhibited only condition (*a*), with the exception of three samples which produced very faint traces of 3-butenyl isothiocyanate in addition to allyl isothiocyanate. Conditions (*b*) or (*c*) defined here were shown only by seeds from India and Pakistan (twenty-one samples). The remaining twenty-six samples received from North America and western Europe had volatile oil production entirely consistent with their described origins from various countries, namely, six samples originating from India and Pakistan showed conditions (*b*) or (*c*), and twenty samples of other origin showed condition (*a*).

The possible amphidiploid origin of *B. juncea* from *B. nigra* (L.) Koch and *B. campestris* L. may account for these variations of volatile oil production, as *B. nigra* has been reported as giving only allyl isothiocyanate[6], which has been confirmed in the present investigations, and *B. campestris* has been found to produce only 3-butenyl isothiocyanate. The correlation with geographical distribution, together with other evidence from investigations of plant and seed morphology, might suggest that natural hybridization to produce *B. juncea* has occurred at different geographical loci.

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Effect of Water Movement on Salt Movement through Tomato Roots

In recent years, there has been renewed debate concerning the effect of water absorption on salt absorption through the roots of intact plants. Salt absorption is regarded by many workers as involving active transport into the xylem by a mechanism dependent on energy released in respiration[1-3]. According to this view, water movement through roots affects salt movement only indirectly by removing salt from the root xylem and thereby producing conditions favourable for increased movement of salt by active transport. On the other hand, several investigators have concluded that, in rapidly transpiring plants, salt moves into the root xylem by mass flow as well as by active transport[4-8].

Estimates of the amount of salt moved by the two processes vary widely. The methods used in the present investigation made it possible to determine accurately that fraction of the total salt moved into the root xylem which was associated with water movement. Root systems of tomato (*Lycopersicum esculentum*, variety Marglobe) started in vermiculite and grown in Hoagland's solution were used in this work.

De-topped root systems were enclosed in a pressure chamber with their stumps projecting through the lid of the chamber. The roots were immersed in aerated half-strength Hoagland's solution which, in some experiments, contained phosphorus-32 as a radioactive tracer. Water was moved through the roots by increasing the air pressure in the chamber. The rate of salt movement through the roots was determined by measuring the volume and salt concentration of the exudate collected from the stumps. Rates of salt movement by active transport were determined by measuring the salt content of root pressure exudate collected before and after pressure applications. The effect of water movement on salt movement was determined by comparing the amount of salt moved through roots when water was moved through them under pressure, with the amount moved by active transport in the absence of external pressure.

The effects of applied pressure on rates of water and salt movement are shown for phosphorus in Fig. 1 and for total salt in Fig. 2. Figs. 1*A* and 2*A* show that, as the rate of water movement through the roots increased with increasing pressure, the phosphorus and total salt concentration of the exudate decreased. The amounts of phosphorus and total salt moved through the root systems increased with increasing pressure and rate of water movement except in the pressure-range of 10–20 lb. The amounts of phosphorus and total salt in root pressure exudate, and the effects of externally applied pressure on their rates of movement, are shown in Figs. 1*B* and 2*B*. The most important effect of increased water movement under pressure was an increase in the total amount of ions moved through the roots. This is shown by the fact that the amounts of phosphorus and total salt in the exudates were always greater during pressure applications than in root-pressure exudates in the absence of pressure. At 30 lb./sq. in., the amounts of phosphorus and total salt moved through the roots were 2·0 times and 2·4 times, respectively, the amounts moved by active transport in the absence of pressure. The amounts of phosphorus and total salt moved under pressure did not increase in proportion to the increased rates of water movement.

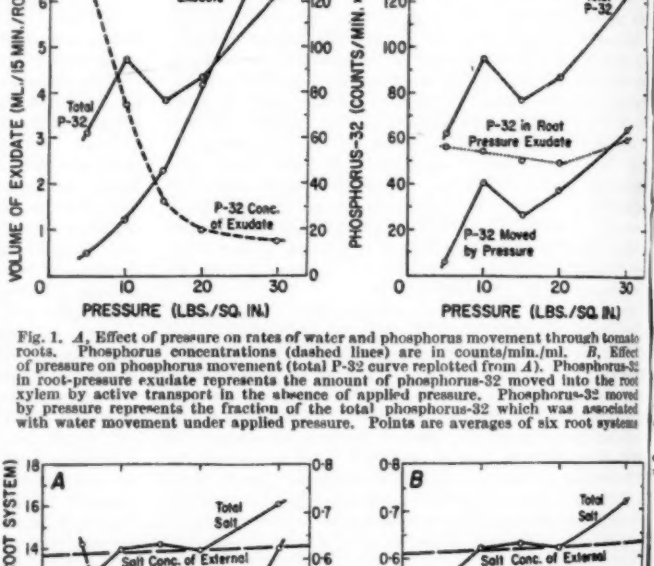

Fig. 1. *A*, Effect of pressure on rates of water and phosphorus movement through tomato roots. Phosphorus concentrations (dashed lines) are in counts/min./ml. *B*, Effect of pressure on phosphorus movement (total P-32 curve replotted from *A*). Phosphorus-32 in root-pressure exudate represents the amount of phosphorus-32 moved into the root xylem by active transport in the absence of applied pressure. Phosphorus-32 moved by pressure represents the fraction of the total phosphorus-32 which was associated with water movement under applied pressure. Points are averages of six root systems

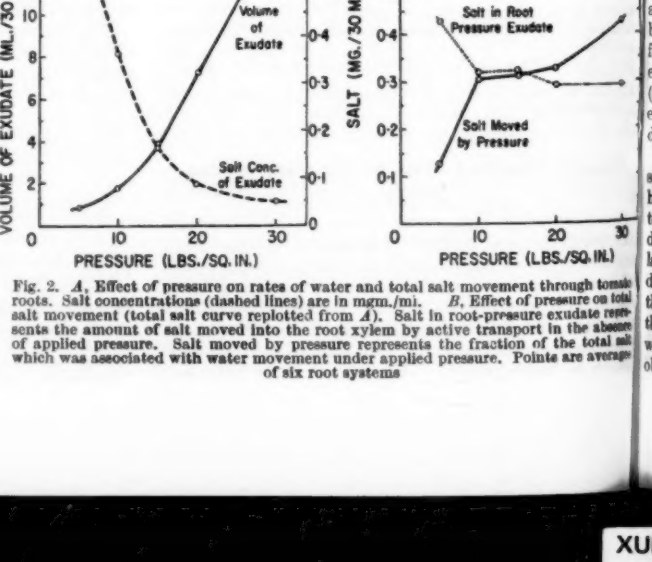

Fig. 2. *A*, Effect of pressure on rates of water and total salt movement through tomato roots. Salt concentrations (dashed lines) are in mgm./ml. *B*, Effect of pressure on total salt movement (total salt curve replotted from *A*). Salt in root-pressure exudate represents the amount of salt moved into the root xylem by active transport in the absence of applied pressure. Salt moved by pressure represents the fraction of the total salt which was associated with water movement under applied pressure. Points are averages of six root systems

Also, the salt concentration of exudates obtained under pressure usually was less than the concentration of the external solutions, indicating the existence of a barrier in the roots which prevents free movement of ions into the xylem.

These results show that rapid water movement through the roots greatly increases the rate of salt movement through the roots. At 30 lb./sq. in., more than 50 per cent of the salt moved through the roots was associated with water movement. The manner in which increased water movement causes increased salt movement, however, is less certain. As the rate of water movement through the roots increases, salt movement into the xylem by active transport may be accelerated by the development of a steeper salt concentration gradient across the root cells from the cortex to the xylem. In addition, the change in the slopes of the total phosphorus and total salt curves at pressures of 15 and 20 lb./sq. in. suggests that the permeability of the roots to ions increased when higher pressures were applied. Some ions might then be carried directly with water into the xylem. An increase in ion permeability of root cells with an increasing pressure gradient across them was reported by Brouwer[7].

The results of these experiments indicate that at low rates of water movement salt movement is primarily by active transport. However, at higher rates of water movement salt movement into the xylem probably occurs by both active transport and mass flow.

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Freeze-drying of Bovine Spermatozoa

SINCE 1956, increased attention has been given to techniques for freeze-drying bovine spermatozoa as a method for long-term semen preservation[1–5]. In one approach[1–3], spermatozoa were dried in vacuum after being diluted, glycerolated, equilibrated and slowly frozen. Low survival-rates and frequent failures were encountered. Leidl[3] suggested three possible causes: (*a*) concentration of glycerol to toxic levels; (*b*) excessive removal of water; (*c*) morphological damage (occasional broken mid-pieces).

In a second approach, Yushchenko[5] freeze-dried spermatozoa suspended in a mixture of 'Freon' and heptane to circumvent the harmful effects of exposure to a eutectic mixture and to eliminate mechanical damage resulting from crystallization. After glycerolation and equilibration in a synthetic medium, the diluted semen was centrifuged in test-tubes containing the 'Freon'–heptane mixture. The spermatozoa were thereby transferred to the non-polar medium in which they were freeze-dried. With this approach he observed 15–20 per cent revival of spermatozoa.

A third technique was described by Meryman and Kafig[4] in which no glycerol was used. Semen diluted in heated milk or egg yolk-citrate was taken up on a 1 × 3 in. sheet of nylon gauze which was suspended in a desiccating chamber connected to a high-capacity vacuum pump (27 cu. ft./min.). Freezing-rate was controlled by constricting the pumping orifice. Since the specimen presented a high surface area to volume ratio, very rapid drying was obtained. They reported 40–50 per cent spermatozoan motility following reconstitution.

These three approaches have been tested in this laboratory and no set of conditions resulted in successful freeze-drying of bovine spermatozoa. The earliest attempts involved diluted semen containing glycerol; occasional motile cells and many tailless sperm and broken or bent mid-pieces were observed. In addition to the deleterious effects suggested by Leidl[3], semen dried in the presence of glycerol resulted in a waxy residue which could not be reconstituted readily.

The procedure of Yushchenko[5] could not be duplicated in that the transfer of spermatozoa from the polar to the non-polar medium was not accomplished. When the two-phase system was centrifuged, the spermatozoa were still completely enveloped by the glycerol-containing polar medium after passing into the 'Freon'–heptane mixture. As expected, freeze-drying this suspension produced the characteristic waxy residue pointed out previously and also resulted in severe morphological damage. Incorporation of surface-active agents such as 'Tweens', 'Carbowaxes', and lecithin did not aid the transfer of cells from the polar to the non-polar phase.

In the original work of Meryman and Kafig[4] a mechanical vacuum pump with a free-air pumping speed of 27 cu. ft./min. was used. Later, H. T. Meryman (personal communication, 1959) suggested that a pump of larger capacity would provide greater versatility in the present work. On this basis, a mechanical pump of 130 cu. ft./min. capacity was used in attempts to confirm their findings. Samples were frozen and dried in a 4 × 9-in. glass desiccating chamber connected to the pump by a 3-in. vacuum line. The line was 47 in. long with one 90° bend. A 3-in. globe valve was inserted in the line 16 in. from the desiccating chamber to control cooling-rate, ultimate temperature of the frozen specimen, and drying-rate When full pumping capacity was utilized with this system (valve wide open), diluted semen suspended on a nylon gauze could be cooled to − 50° C. solely by evaporation and sublimation.

More than 250 trials were made using a wide range of cooling- and drying-rates by adjusting the size of the opening in the 3-in. valve. Heated skim milk diluent was used in most of the trials; however, egg yolk-citrate and undiluted semen also were tested. Freezing- and drying-rates comparable with those of Meryman and Kafig[4] could be achieved readily. However, they did not report quantitative data on desiccating chamber pressures and residual moisture of the specimen, and failure in the present work may be due to inability to satisfy these conditions. In a few trials, occasional motile spermatozoa were obtained. Further investigation indicated that the motile cells usually were associated with a thickened layer of material which accumulated at the bottom of the vertically suspended nylon gauze prior to freezing. The innermost part of this material was not dry and seemed to be the source of the motile spermatozoa. When the membrane was kept under vacuum

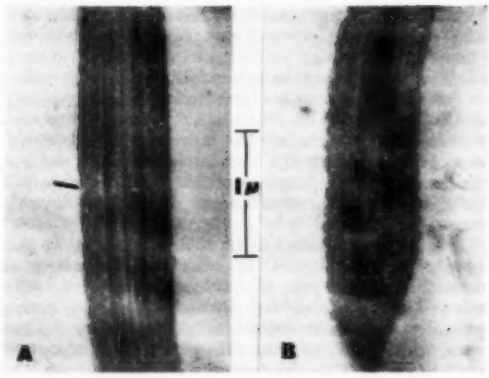

Fig. 1. Ultrastructure of the mid-piece of bull spermatozoa showing (*A*) the presence of the cytoplasmic membrane (arrow) in a control, and (*B*) its absence in a freeze-dried specimen. (× *c.* 19,700)

long enough to assure complete drying of the semen, no motile spermatozoa were observed.

In contrast to cells freeze-dried in the presence of glycerol, both diluted and undiluted spermatozoa dried by the latter technique appeared morphologically normal when examined under a phase contrast microscope (× 660). Next, the ultrastructure of freeze-dried spermatozoa was studied with the electron microscope using thin sections. Freshly collected, pooled ejaculates of undiluted semen were freeze-dried on nylon mesh gauze in vacuum and reconstituted in a 2·9 per cent sodium citrate solution. Control spermatozoa were taken up on a nylon gauze and then resuspended in 2·9 per cent sodium citrate without freeze-drying or exposure to vacuum. Spermatozoa were centrifuged from the citrate solution into a loose pellet which was fixed with Caufield's modification[6] of Palade's fixative[7]. 'Araldite' was used as the embedding material and sectioning was performed with a diamond knife on a Porter–Blum ultramicrotome. Electron micrographs revealed that spermatozoa subjected to freeze-drying and reconstitution usually lacked the outer or cytoplasmic membrane (Fig. 1*B*), while control spermatozoa usually displayed an intact membrane (Fig. 1*A*). The presence or absence of the membrane was most apparent in longitudinal sections of the middle piece or principal piece. It is not known whether the same results would have been obtained if the spermatozoa had been diluted before freeze-drying. This possibility is being investigated.

The electron microscope also was used to examine undiluted spermatozoa frozen under vacuum to − 20° C. and thawed in citrate solution at room temperature and undiluted spermatozoa rapidly cooled to 0° C. by vacuum without freezing. As observed in freeze-dried spermatozoa, the cytoplasmic membrane of frozen and thawed cells usually was lacking. Spermatozoa rapidly cooled under vacuum without freezing generally possessed intact membranes.

These observations on bull spermatozoa suggest that alteration or destruction of the cytoplasmic membrane represents one possible deleterious effect on survival after either freeze-drying and reconstitution, or freezing and thawing in the absence of glycerol. It is not known whether these treatments cause death and subsequent loss of the cytoplasmic membrance or vice versa. The importance of the cytoplasmic membrane and factors which affect its integrity are being investigated.

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Precipitating Antibodies in the Carpet Snake against Parasitic Nematodes

To our knowledge very little attention has been paid to the formation or presence of precipitating antibodies in the cold-blooded vertebrates. Indications of active antibody formation have nevertheless been reported in frogs, turtles and fish; however, these estimations were made using either agglutination or anaphylaxis[1]. This communication is a survey of naturally occurring antibodies in the carpet snake (*Morelia spilotes variegatus*) which react positively with the body fluids of their nematode parasites, *Amplicaecum robertsi*, *Ophidascaris* sp., and *Polydelphis* sp., in interfacial precipitin ring tests.

The antigen was collected by puncturing the cuticle of these nematodes and drawing the fluids up a fine glass capillary; the body fluids from approximately fifty worms being pooled, in each case, to give sufficient antigen for these experiments. Blood was collected from the aortas of twelve snakes at autopsy, defibrinated and the serum stored frozen. Table 1 shows the results of interfacial precipitin ring tests using these sera versus the body fluids of the three snake nematodes and the presence or absence of the particular infection in the snakes at the time of autopsy.

In general these results indicate that circulating, precipitating antibodies exist in the sera of carpet snakes which react with the antigens of nematodes infecting them, and that the presence of antibody is probably related to the presence of the particular infection ($P \sim 14$ per cent). Certain expected discrepancies exist in these results, namely, antibodies are present in the absence of infection and infections also occur without detectable antibody. These discrepancies can arise since we do not know either the beginning or the duration of these infections or the number of previous infections (we have noticed that carpet snakes may regurgitate their partially digested food with some of their intestinal nematodes, which may explain how some infections are lost). The possibility also exists that the circulating antibodies are non-specific. To test the specificity of these antibodies the various sera were tested against the body fluids of *Ascaris suum*, from the pig, as antigen. It was found that three of the twelve sera gave positive reactions. This apparent lack of specificity in these

Table 1. THE PRESENCE OR ABSENCE OF ANTIBODY AND INFECTION IN CARPET SNAKES (*Morelia spilotes variegatus*) NATURALLY INFECTED WITH THREE PARASITIC NEMATODES

Parasitic nematode	Carpet snake A		B		C		D		E		F		G		H		I		J		K		L	
	Ab	Inf	Ab	Inf	Ab	Inf	Ab	Inf	Ab	Inf	Ab	Inf	Ab	Inf	Ab	Inf	Ab	Inf	Ab	Inf	Ab	Inf	Ab	Inf
Amplicaecum robertsi	+	+	+	+	+	−	−	−	+	+	+	+	+	+	+	+	+	−	+	−	−	−	+	−
Polydelphis sp.	−	−	+	+	+	+	−	−	−	−	+	+	+	−	−	−	+	−	+	+	+	+	+	+
Ophidascaris sp.	−	+	+	−	+	+	+	−	+	+	−	+	+	−	+	+	+	+	+	+	−	+	+	+
Ascaris suum	+		+		−		−		−		+		−		−		−		−		−		−	

Ab, antibody; Inf, infection.

three cases may be accounted for by the presence of antibodies versus Forssman antigen known to exist in certain helminths[2]. However, the carpet snake must also be able to produce specific antibodies against antigens from its three nematode parasites since the sera negative to *A. suum* gave positive reactions against these worms ($P \sim 1$ per cent). Further work is being done on the natural antibodies found in the carpet snake.

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Fibre Follicles in Romney Ewes

ORWIN has reported[1] the occurrence of what he describes as 'branching follicles' in skin from two-year-old New Zealand Romney ewes: in his communication there appears to be some confusion concerning the evidence required to demonstrate conclusively whether follicles are branched, that is, have outgrowths of derived follicles, or are separate follicles which have coalesced. Orwin's photographs show more than one fibre in a common sheath at sebaceous-gland level and at a higher level; this, however, in itself is not adequate evidence that one follicle has been derived from another by branching. These follicles may equally well have been separately initiated and coalesced so that the fibres come to lie in a common sheath and a common follicular mouth.

Auber[2] has indeed demonstrated the occurrence of wool follicles coalescing at a level below that of the sebaceous glands. Serial skin sections at levels which include lower levels than that of the sebaceous glands should be made, the follicles traced up and observations confirmed by 'vertical' sections, before deciding whether a follicle has been derived from another one by branching or has coalesced with it. I do not suggest that follicles derived by branching do not occur in breeds of sheep other than the merino; however, in many thousands of serial skin sections from English Romney sheep and lambs that I have examined at all levels I have sometimes seen coalesced follicles, but have not seen truly branched follicles.

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Localization of Inhibitors of Neural Differentiation in Chick Embryos

IN testing the hypothesis of specific inhibition in cellular differentiation, I have shown[1] that early chick embryos, when cultured on a nutrient medium which has incorporated in it supernatants of adult and embryonic brain extracts, displayed homologous brain defects in a significant number of cases. Since the preparation of subcellular components has become a standard technique for problems of cellular chemistry (see review by Allfrey[2]), this investigation was prompted with the hope of localizing the inhibitory material in the crude brain extracts to a particular cell fraction. Accordingly, chick brains were homogenized and subjected to differential centrifugation in order to obtain mitochondrial, microsomal, and soluble protein fractions. Earlier investigations of Braverman[3] and Lenicque[4] showed that the nuclear fraction is not involved. The procedure used for culturing chick embryos was that of Spratt[5]. Since I have found[1] that stages 4 and 5 (ref. 6) are the crucial stages for treatment with brain extracts, the assay for inhibitory activity was restricted to the use of these stages.

Whole brains from recently hatched chicks totalling 10–15 gm. were homogenized in 2 vol. (w/v) of 0·25 *M* sucrose–0·004 *M* calcium chloride in a Potter–Elvehjem homogenizer. The nuclei and cellular fragments were sedimented in an International refrigerated centrifuge at 700*g* for 10 min. The supernatant was then subjected to 15,000*g* for 30 min. in a Servall centrifuge. This resulted in a white pellet of mitochondria, which was dispersed in phosphate-buffered chick Ringer, *p*H 7·6–7·8. The supernatant was centrifuged at 105,000*g* for 60 min. in a Spinco *L* ultracentrifuge, rotor No. 40. This yielded a microsomal pellet, and a supernatant consisting of the soluble protein fraction. The former was dispersed in chick Ringer, while the soluble protein fraction was subjected to 18–24 hr. dialysis against chick Ringer. All preparations were carried out in the cold room at a temperature of 0–2° C.

Aliquots of the samples were taken for quantitative estimations of protein nitrogen using the Kjeldahl method. The nutrient medium composed of 0·5 per cent agar, albumen extract, and subcellular fraction was made up in the ratio of 2:1:1. The concentration used in these experiments was 75–90 μgm. of protein nitrogen of subcellular component per c.c. of culture medium.

Controls using a myosin preparation obtained through the courtesy of Dr. Yujiro Hayashi and adjusted to the equivalent concentrations as with the subcellular fractions in terms of protein nitrogen were used. It was intended that the myosin sample, obtained from the leg muscles of the same chicks which were the sources for the brains, would serve as a control of mesodermal origin. According to Dr. Hayashi, the myosin samples, prepared by a modification of the method of Hasselbach and Schneider[7], showed a single peak when examined by both ultracentrifugal and electrophoretic methods. Before use,

Table 1. Effect of Subcellular Fractions of Brain on Neural Differentiation

	Subcellular fraction: Mit.	Mic.	Sol. protein	Myosin
Total No. of explants:	53	67	80	45
Kinds of defects:				
Normal	41 (77%)	54 (81%)	15 (19%)	8 (18%)
General defect	7 (13%)	5 (7%)	6 (8%)	—
No development	5 (9%)	5 (7%)	10 (11%)	5 (11%)
Brain limited defect	0 (0%)	3 (4%)	49 (61%)	6 (13%)
Mesodermal and general defect	—	—	—	26 (58%)

the myosin samples were dialysed against chick Ringer solution.

The results of this investigation are presented in Table 1. It indicates that the inhibitory factor is localized in the final supernatant or soluble protein fraction. For a discussion of the classification of defects see ref. 1, since the same system was used in this work. In regard to the myosin controls, at concentrations equivalent to the experimental series, that is, 75–90 μgm. of protein nitrogen, it was found to be highly toxic for the developing embryos, and resulted in all embryos undergoing no development. Consequently, the level has to be lowered considerably, to 15–20 μgm. per c.c. of culture medium. It appeared that the mesodermal defects, which are manifested in circulatory abnormalities, concomitantly lead to generalized defects. Thus, a high number of combined mesodermal and general defects were recorded. This is not surprising in view of the fact that the myosin preparation represents a relatively purified sample, as compared with the fractions obtained from brain material, which are still rather crude preparations. In view of this fact, future work is planned in an effort to characterize and isolate further the inhibitory properties of the soluble protein fraction. In this connexion, fractionation of the soluble protein fraction may yield a more potent and pure source of the specific neural differentiation inhibitor.

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ENTOMOLOGY

An Apparatus for Observations on the Feeding Mechanism of the Flea

Several types of feeding chambers have been devised[1] for *in vivo* observations on the feeding mechanism of blood-sucking arthropods, but these chambers, designed to accommodate relatively large creatures (triatomine bugs, tsetse flies and ticks), have proved to be unsuitable for small insects and acarines, such as fleas, lice and mites. Our interest in the mechanism of disease transmission by the flea led us to construct a small feeding apparatus by means of which we have been able to undertake extensive observations on the uptake of blood by this insect from the tissues of its host.

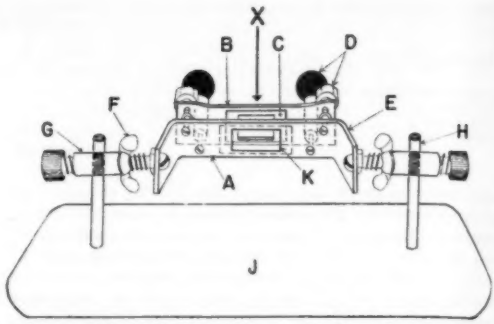

Fig. 1. The feeding apparatus as seen from the microscope

The apparatus (Fig. 1) consists essentially of two apposable, thin, steel plates (*A* and *B*), each with a centrally placed viewing window (*K*).

Plate *A*, 6·3 cm. in length and 1·3 cm. in height, is extended at both extremities to form right-angled extensions (*E*), 1·5 cm. long. Each extension is provided with a horizontal bar (*G*) which fixes into a vertical rod (*H*) attached to a carrying table (*J*). The carrying table is clamped to the reversed mechanical stage of a horizontally placed microscope as described by Griffiths and Gordon[2] for an earlier type of feeding chamber.

Plate *B*, the same size as *A*, is joined to plate *A* by means of two pairs of adjusting screws (*D*). The adjusting screws (the upper pair is threaded through light springs) enable the plates to be apposed and tilted so that their lower edges can be approximated to enclose the ear of the host (Fig. 2).

The viewing windows (*K*), 1·6 cm. × 0·8 cm., are each covered by a coverslip (*L*) of a convenient size held in position by an adhesive applied to the edges of the window.

An anæsthetized mouse is placed on the carrying table. The upper portion of one of its ears, previously treated with a depilatory, is so arranged as to lie over the coverslip on plate *B*, and a strip of foam rubber is then introduced between the plates, one on each side of the window at position *C* (Fig. 1). The adjusting screws are gradually tightened until the lower edges of both plates gently grip the ear, the upper edges being kept separated by the springs of the upper pair of screws. In this position plate *B*, carrying the ear, lies at an angle to plate *A* (which is vertical to the microscope tube). In order that the ear should present a flat microscopic field the apparatus is rotated by unloosening the wing nuts (*F*), until the plates have assumed the position shown in Fig. 2, and the nuts are retightened.

A flea (*N*) anæsthetized with carbon dioxide is then introduced into the chamber, and the upper part is closed off promptly with a strip of foam rubber (*M*). The space enclosed by the lower edges of the plates, the two vertical (*C*) and the horizontal (*M*) strips of foam rubber, forms a completely sealed cell (Fig. 2) containing the tip of the animal's ear and the insect.

We observed, using this simple apparatus, that *Pulex irritans*, *Xenopsylla cheopis*, *Ctenocephalides felis* and *Atyphloceras multidentatus* normally feed, in non-sensitized mice, directly from the lumen of small blood vessels, and thus may be described as capillary feeders. On rare occasions we saw fleas attempting to feed from extravasations of blood resulting from the rupture of vessels, but they never took up any significant quantities of blood from this source.

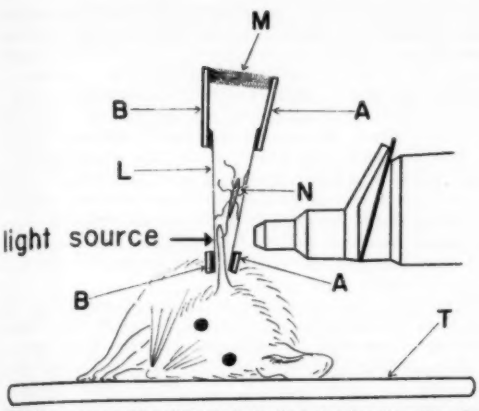

Fig. 2. A cross-section through the feeding chamber at position *X* on Fig. 1 (showing a flea feeding on a mouse's ear) after the apparatus has been rotated so that plate *B*, instead of plate *A*, is vertical to the tube of the microscope

Feeding appears to proceed in this way: the flea introduces its biting fascicle[3]—the paired maxillæ and the epipharynx—into the skin of the host, the maxillæ acting as the cutting organs. Having reached a suitable vessel, usually of small calibre, the tip of the epipharynx enters the lumen, the maxillæ (which form the salivary canals) remaining outside. The flea then sucks up blood, emitting from time to time, as clearly visible puffs, droplets of saliva in the neighbourhood of the puncture in the vessel wall (but not into the vessel lumen). When the meal is completed the fascicle is rapidly withdrawn; there is surprisingly little damage at the point of introduction of the mouthparts into the skin, the withdrawal hæmorrhage being slight or absent.

We wish to acknowledge the assistance of a National Institutes of Health grant, *E*-3571, in carrying out this project and the help of Frank Prince and his colleagues (of C.D.C. station at San Francisco), who kindly provided us with a proportion of the wild and laboratory bred fleas we used in our experiments. Histological material, which confirmed our direct observations, was prepared by Rosalie Gill of the Hooper Foundation, to whom we are indebted.

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Intestinal Proteinases in an Insect, *Calliphora vomitoria* L.

The properties of the proteolytic enzymes occurring in extracts of insect guts are generally thought to resemble those of vertebrate trypsin[1,2]. Only proteinases of alkaline type, with maximum activity about *p*H 8, have been recorded in *Calliphora* adults[3], *Lucilia* larvæ[4], *Musca* larvæ[5], *Stomoxys* adults[6], and *Calliphora* larvæ[7]. The only workers to suggest that proteinases in Diptera–Cyclorrhapha do not conform to the general theory, Greenberg and Paretsky[8], reported two distinct proteinases functioning in every stage of the life-cycle of *Musca* with *p*H optima at *p*H 3 and *p*H 8–9.

It has now been established by two techniques that a proteolytic enzyme (or enzymes) with an activity peak about *p*H 3 is produced by cells of the mid-gut of adult females of the blowfly species *C. vomitoria*.

Proteolytic activity of aqueous extracts of mid-guts of flies fed on meat was determined *in vitro*, over a selected *p*H range, by a colorimetric method. Sheep hæmoglobin was used as substrate, prepared in citrate–phosphate buffer, and mixtures of buffered substrate and gut extract were incubated for 3 hr. at 38° C. After removal of undigested protein, by precipitation (with 10 per cent trichloracetic acid) and centrifugation, the mixture was made alkaline by the addition of sodium carbonate. The presence of liberated tyrosine, and tryptophan, was demonstrated by the addition of Folin–Ciocalteau reagent, the intensity of the blue colour which develops being used as a measure of the degradation of the substrate. Direct readings of colour intensities were made with an *EEL* portable colorimeter (*O.R.*1 light filter) exactly 5 min. after the addition of the reagent. The graph in Fig. 1 was prepared from means of three determinations of proteolytic activity at thirteen *p*H-levels between *p*H 2·5 and *p*H 8·5. The results indicate a high concentration of an enzyme (or enzymes) in gut extracts with an optimum *p*H about *p*H 3 and a lower concentration of an enzyme (or enzymes) with an optimum *p*H at least as high as *p*H 8·5. Controls were prepared at several *p*H-levels and tested for free tyrosine, etc., immediately after preparation, without incubation. Readings for these controls approximated to those obtained from digests between *p*H 5·5 and *p*H 7, indicating that there is little enzyme activity within that *p*H-range. Further tests

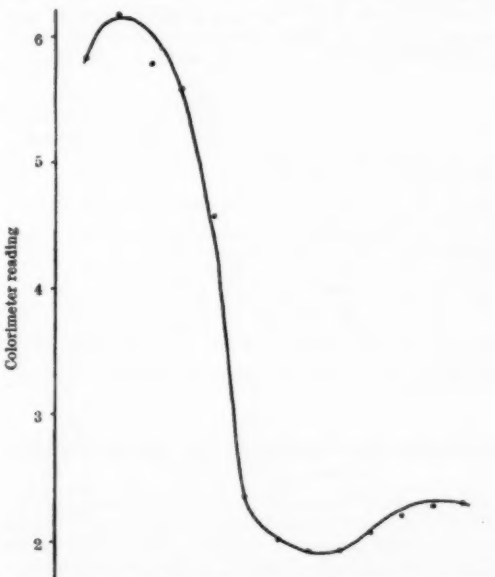

Fig. 1

carried out at *p*H 3 with distilled water substituted for enzyme extract showed that the high reading at that *p*H was not due to autolysis of the substrate.

In the second demonstration of proteolytic activity at low *p*H, mid-guts were dissected from 5-day old flies which had fed on meat. The guts were split longitudinally and the peritrophic membrane tube and contents removed. Each gut was then divided into three parts corresponding to the three zones, fore-mid-gut, mid-mid-gut and hind-mid-gut, the contents of which are buffered (as shown by Waterhouse's range-indicator method[9]) at *p*H 5, *p*H 3·6, and *p*H 8. These fractions were then laid on the surface of a thin milk-agar plate of the type normally used for bacteriological work. The milk–agar medium, which contained thiomersalate to inhibit bacteria, was either buffered at *p*H 3·6 or *p*H 8. Preparations were incubated for 24 hr. at 38° C. In the low *p*H plates a wide zone of clearing developed around the mid-mid-gut. There was also some evidence of an enzyme active at low *p*H diffusing from the last part of the fore-mid-gut in smaller quantity than that from the mid-mid-gut. Clearing occurred in the immediate vicinity of the hind-mid-gut in *p*H 3·6 plates, but this appeared to be restricted to the range of the gut cells' own buffering effect beyond which the hind-mid-gut enzyme was obviously inactive. In *p*H 8 plates a wide zone of clearing developed around the hind-mid-gut. Thus the results of the quantitative tests have been confirmed and it has been shown that the proteinase (or proteinases) active at low *p*H in the gut of *C. vomitoria* is principally produced by the cells of that zone the *p*H of which is adjusted to suit such an enzyme.

Female blowflies require protein in their diet in order to produce eggs. Males have no such need, but it is interesting to note that they will feed readily on meat, and the milk-agar plate method indicates the presence of the same enzymes in male as in female intestines.

We thank Drs. J. R. Norris and W. R. Rees of this University for valuable discussions and Dr. Ellen Thomsen, Copenhagen, for the initial suggestions.

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Home and Distribution of the House Cricket *Acheta domesticus* L.

The house cricket, *Acheta domesticus*, is generally believed to have moved into Europe and North America from arid and semi-arid areas of northern Africa or south-western Asia[1]. However, during my work on *Acheta domesticus* and related species, I have found evidence which suggests that the question regarding the native country and distribution of *A. domesticus* is more complicated.

Colour and size are unreliable taxonomic characters for separating closely related species of crickets, and the species of the *domesticus* group are remarkably uniform in their outward appearance. However, among the male specimens from various parts of the world, identified as *Acheta domesticus* by Chopard, Uvarov, Dirsh, Kevan, Gurney and other specialists, I have found no less than five species which have no significant external morphological differences but can be clearly separated on the basis of their internal male genitalia. Indeed, two of these species have been experimentally shown to be reproductively isolated[2], but the anatomical reasons for this have only now become clear.

The first step to clear the confusion regarding the taxonomy of the house cricket is to fix the name *Acheta domesticus* L. for one of the five species distinguished in the course of the present studies. The name *A. domesticus*, described from Sweden, should be restricted to the house cricket commonly found in Europe and North America, including the Canadian strain[2]. The males determined on the basis of genitalia were compared with *domesticus* specimens from Sweden, and both sexes taken from the same laboratory-bred stock are being re-described.

Uvarov[1] described on the basis of colour and size *Acheta meridionalis* from Khartoum as a sub-species of *A. domesticus*, and Kevan[3] considered it a wild southern form of the same, but the examination of male genitalia shows that *A. meridionalis* (Uvarov) is a distinct species, known to me also from West Aden Protectorate, Egypt and Fezzan. The examination of the male genitalia shows also that the species referred to earlier as the Pakistani strain[2] is *Acheta hispanicus* Rambur, which I am able to record also from Morocco, Baghdad and West Pakistan. Three more distinct species of the *domesticus* group are known to me from Egypt, East Africa and India.

The specimens of all the species referred to above are preserved in the British Museum (Natural History), London, and their full descriptions, including a re-description of *Acheta domesticus* L. with full synonymy, are ready for publication. Once the identities of the species previously confused with *A. domesticus* are established, it may become possible to build up a better picture of the distribution of the European house cricket (*A. domesticus*), and to determine its native country with greater certainty. The correct identification of these species is also of great economic importance as at least two of them (*A. domesticus* and *A. hispanicus*) are serious pests of irrigated crops in the Indo-Pakistan sub-continent[4], and with the extension of canal irrigation in the arid and semi-arid regions their potential danger continues to increase. I shall be glad to receive material on these crickets either from houses or collected in the field for further studies.

This work was carried out while I held a research fellowship of the South East Asia Treaty Organization and I am grateful for it, and also to Sir Boris Uvarov and Dr. V. M. Dirsh for their advice and help.

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FORTHCOMING EVENTS

*(Meetings marked with an asterisk * are open to the public)*

Monday, December 11

BRITISH SOCIETY FOR THE PHILOSOPHY OF SCIENCE (in the Joint Staff Common Room, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. L. S. Penrose, F.R.S.: "A Theory of Self-reproducing Mechanisms".

UNIVERSITY OF LONDON (in the Anatomy Theatre, University College, Gower Street, London, W.C.1), at 6 p.m.—Prof. W. K. Estes: "Current Foci of Research in Mathematical Behaviour Theory".* (Last of three Special University Lectures.)

INSTITUTION OF ELECTRICAL ENGINEERS, LONDON GRADUATE AND STUDENT SECTION (at Savoy Place, London, W.C.2), at 6.45 p.m.—Mr. G. S. C. Lucas, O.B.E.: "The Professional Engineer and an Expanding Industry" (Presidential Address).

Tuesday, December 12

ZOOLOGICAL SOCIETY OF LONDON (at the Zoological Gardens, Regent's Park, London, N.W.1), at 5 p.m.—Scientific Papers.

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS AND COMMUNICATIONS SECTION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. L. Lewin: "Diversity Reception and Automatic Phase Reception".

UNIVERSITY OF LONDON (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Dr. I. D. P. Wootton: "Retention of Aromatic Compounds in Acute Renal Failure".* (Last of seventeen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation.)

INSTITUTION OF MECHANICAL ENGINEERS, AUTOMOBILE DIVISION (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Mr. J. G. G. Hempson: "Instrumentation Problems in Internal Combustion Engine Development".

UNIVERSITY OF LONDON (in the Beveridge Hall, Senate House, University of London, London, W.C.1), at 6 p.m.—Sir Charles Snow: Birkbeck College Foundation Oration.

INSTITUTE OF WOOD SCIENCE (at Carpenters Hall, Throgmorton Avenue, London, E.C.2), at 7 p.m.—Dr. A. C. Copisarow: "The Role of Forest Products Research in National Economy".

Tuesday, December 12—Wednesday, December 13

BRITISH BIOPHYSICAL SOCIETY (at the Royal Institution, Albemarle Street, London, W.1)—Symposia on "The Structure of Globular Proteins" and "The Function of Proteins", also a session on contributed papers.

Wednesday, December 13

ROYAL STATISTICAL SOCIETY (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.15 p.m.—Mr. I. D. Hill: "Sampling Inspection and Defence Specification DEF-131".

INSTITUTE OF FUEL (at the Royal United Service Institution, Whitehall, London, S.W.1), at 5.30 p.m.—Mr. A. Sherry: "Power Station Optimization".*

INSTITUTION OF ELECTRICAL ENGINEERS, SUPPLY SECTION (in conjunction with the BRITISH NUCLEAR ENERGY CONFERENCE, at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. J. Henderson, Mr. G. F. Kennedy and Dr. K. J. Wootton: "Electrical Aspects of Hunterston Nuclear Generating Station".

ROYAL SOCIETY OF ARTS (at John Adam Street, Adelphi, London, W.C.2), at 6 p.m.—Dr. E. S. Hedges: "Tin—Its Contribution to Social Development".

SOCIETY OF CHEMICAL INDUSTRY, FOOD GROUP—NUTRITION PANEL (at 14 Belgrave Square, London, S.W.1), at 6.15 p.m.—Meeting on "Foods Low in Carbohydrates".

SOCIETY FOR ANALYTICAL CHEMISTRY, MICROCHEMISTRY GROUP (at "The Feathers", Tudor Street, London, E.C.4, at 6.30 p.m.—Discussion Meeting.

SOCIETY OF INSTRUMENT TECHNOLOGY, CONTROL SECTION (at Manson House, 26 Portland Place, London, W.1), at 7 p.m.—Mr. P. K. M'Pherson: "Application of Complex Plane Methods to System Design".

BRITISH PSYCHOLOGICAL SOCIETY, MEDICAL SECTION (at the Royal Society of Medicine, 1 Wimpole Street, London, W.1), at 8.30 p.m.—Mr. Stephen Black: "Hypnosis in Physiological Research".

Thursday, December 14

SOCIETY OF CHEMICAL INDUSTRY, ROAD AND BUILDING MATERIALS GROUP (at 14 Belgrave Square, London, S.W.1), at 2.30 p.m. and 5.30 p.m.—Special Meeting on "New Test Methods".

IMPERIAL COLLEGE OF SCIENCE AND TECHNOLOGY (in the Physics Building, Prince Consort Road, London, S.W.7), at 5.30 p.m.—Sir Patrick Linstead, F.R.S.: "The Prince Consort and the Founding of the Imperial College" (Special Lecture to commemorate the Centenary of the Death of H.R.H. The Prince Consort.)

INSTITUTION OF ELECTRICAL ENGINEERS, UTILIZATION SECTION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Dr. E. R. Laithwaite: "Oscillating Machines—Synchronous and Asynchronous"; Dr. E. R. Laithwaite and Mr. R. S. Mamak: "An Oscillating Synchronous Linear Machine".

BRITISH INSTITUTION OF RADIO ENGINEERS, TELEVISION GROUP (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 6 p.m.—Symposium on "Constant Luminance Colour Television".

SOCIETY FOR ANALYTICAL CHEMISTRY, BIOLOGICAL METHODS GROUP (in "The Feathers" Restaurant, Tudor Street, London, E.C.4), at 6.30 p.m.—Seventeenth Annual General Meeting, followed by a Discussion Meeting on "The Assessment of Anti-Aretheroscleroticcs".

RADAR AND ELECTRONICS ASSOCIATION (in the Lecture Hall of the Royal Society of Medicine, John Adam Street, Adelphi, London, W.C.2) at 7 p.m.—Dr. J. Gough and Prof. F. Landgrebe: "Loudspeaker Enclosures".

CHEMICAL SOCIETY (in the Lecture Theatre of the Royal Institution, Albemarle Street, London, W.1), at 7.30 p.m.—Prof. C. E. H. Bawn, F.R.S.: "Stereospecific Polymerisation" (Liversidge Lecture).

ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE (at Manson House, 26 Portland Place, London, W.1), at 7.30 p.m.—Dr. F. I. C. Apted: "Sleeping Sickness in Tanganyika, Past, Present and Future".

Friday, December 15

SOCIETY FOR ANALYTICAL CHEMISTRY (joint meeting with the INFRA RED DISCUSSION GROUP, at King's College, Strand, London, W.C.2), at 11.10 a.m.—Meeting on "Applications of Infra Red Spectroscopy to Quantitative Analysis".

Saturday, December 16

BIOCHEMICAL SOCIETY (at the Wolfson Institute, Postgraduate Medical School of London, Ducane Road, London, W.12), at 10.30 a.m.—Scientific Papers and Demonstrations.

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

ASSISTANT EXPERIMENTAL OFFICER or EXPERIMENTAL OFFICER (preferably with a university degree) AT THE FRESHWATER FISHERIES LABORATORY, Department of Agriculture and Fisheries for Scotland, Pitlochry—Establishment Officer, Room 73, Department of Agriculture and Fisheries for Scotland, St. Andrew's House, Edinburgh 1 (December 15).

ASSISTANT INSPECTOR (male, graduate in zoology, etc.) OF FISHERIES in the Department of Lands, Dublin—The Secretary, Civil Service Commission, 45 Upper O'Connell Street, Dublin 1, Republic of Ireland (December 15).

VERTEBRATE BIOLOGIST (with ethological training or experience), for a research post in a Mental Hospital—The Secretary, Birmingham Regional Hospital Board, 10 Augustus Road, Edgbaston, Birmingham 15 (December 16).

ASSISTANT IN THE DEPARTMENT OF GENETICS, for general duties and particularly to assist on work on the genetics of fungi—The Secretary, John Innes Institute, Bayfordbury, Hertford, Herts (December 27).

LECTURER or ASSISTANT LECTURER IN PHYSICS—The Principal, Bede College, Durham (December 31).

LECTURER or SENIOR LECTURER IN GENETICS at the University of Melbourne, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, December 31).

READER (with special qualifications in educational psychology) IN EDUCATION at the University of Queensland, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Brisbane and London, December 31).

HEAD (with high academic qualifications and experience in research and in teaching to university degree level) OF THE DEPARTMENT OF PHYSICS—The Clerk to the Governors, Chelsea College of Science and Technology, Manresa Road, London, S.W.3 (January 1).

LECTURER (with a registrable veterinary qualification and postgraduate experience in bacteriology) IN BACTERIOLOGY in the School of Veterinary Medicine—The Registrar, Trinity College, Dublin 2, Republic of Ireland (January 1).

BIOPHYSICIST (post-doctoral standing) IN THE ELECTRON MICROSCOPE SECTION, to take part in an investigation of the biological applications of the electron scanning microanalyser (X-ray microspectrometer)—The Secretary, Department of Physics, Cavendish Laboratory, Free School Lane, Cambridge (January 5).

CHAIR, and LECTURER or ASSISTANT IN PHILOSOPHY at the University of Malaya, Singapore—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (January 15).

LECTURER (preferably with a special interest in historical geography) IN GEOGRAPHY at the University of Melbourne, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (January 15).

ASSOCIATE PROFESSOR IN EXPERIMENTAL PATHOLOGY at the University of Sydney, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, January 17).

LECTURER (preferably with some experience in steroid and/or carbohydrate chemistry) IN ORGANIC CHEMISTRY at the University of Sydney, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, January 17).

CHAIR OF AGRICULTURAL CHEMISTRY IN THE DEPARTMENT OF AGRICULTURE, University of Sydney, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, January 31).

CHAIR OF COLOUR CHEMISTRY AND DYEING—The Registrar, The University, Leeds 2 (January 31).

LECTURER (trained and experienced teacher with a degree in chemistry or biology) IN EDUCATION at the University of Hong Kong—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Hong Kong and London, January 31).

LECTURER (with at least an honours degree or equivalent qualifications, some postgraduate research experience, and preferably interests in mammalian or human biochemistry) IN BIOCHEMISTRY in the School of Biological Sciences, University of New South Wales—The Agent-General for New South Wales, 56–57 Strand, London, W.C.2 and The Appointments Section, University of New South Wales, Box 1, Post Office, Kensington, New South Wales, Australia (January 31).

OFFICIAL FELLOW AND LECTURER (mathematician or theoretical physicist) IN MATHEMATICS—The Vice-Provost, Worcester College, Oxford (January 31).

SENIOR RESEARCH ASSISTANT (with a higher degree in chemistry or biochemistry, and several years experience in biochemical research) IN BIOCHEMISTRY, to be engaged full time in a research programme in the field of protein chemistry—The Director of Research, St. Vincent's School of Medical Research, Victoria Parade, Fitzroy, N.6, Victoria, Australia (February 28).

ASSISTANT, ASSOCIATE OR FULL PROFESSOR OF PSYCHOLOGY and an ASSISTANT PROFESSOR OF PSYCHOLOGY—Prof. J. R. Royce, Head, Department of Psychology, University of Alberta, Edmonton, Alberta, Canada.

ASSISTANT PROFESSOR OF PHYSICS—Prof. C. E. Challice, Department of Physics, University of Alberta, Calgary, Alberta, Canada.

ASSOCIATE PROFESSOR OR ASSISTANT PROFESSOR IN THE DEPARTMENT OF PHYSICS—Head of the Department of Physics, Victoria College, Victoria, B.C., Canada.

HISTORICAL ARCHÆOLOGIST (graduate with specialization in archæology, approximately four years experience in conducting archæological investigations, including the excavation of historical ruins) in the Department of Northern Affairs and National Resources, Louisbourg, Nova Scotia, Canada, to undertake all archæological excavations in connexion with the restoration of the Fortress of Louisbourg National Historic Park—Civil Service Commission of Canada, Ottawa, Ontario, Canada.

MASTER TO TEACH MATHEMATICS to public school scholarship standard—The Headmaster, Lathallan School, Montrose, Angus.

PHYSICISTS OR ELECTRICAL ENGINEERS (preferably with experience in one or more of the following fields: high vacuum; R.F. techniques (75 mc./s.); high power rectifier and pulse circuits; general electronic instrumentation and controls) IN THE DEPARTMENT OF PHYSICS, to assist with the construction and commissioning of a large heavy ion linear accelerator—The Assistant Director, Linear Accelerator Laboratory, Physics Department, The University, Manchester.

RESEARCH ASSISTANT (with a degree in engineering and some knowledge of mechanical vibration) IN THE DEPARTMENT OF ENGINEERING, for work on certain problems related to the vibration of turbine blades—The Secretary, The University, Old College, South Bridge, Edinburgh.

RESEARCH FELLOW (recent or prospective Ph.D. graduate in biochemistry, physiology, nutrition or allied subjects) IN THE FIELD OF NUTRITION IN THE DEPARTMENT OF PHYSIOLOGY AND BIOCHEMISTRY—The Secretary and Registrar, The University, Southampton.

SCIENTIFIC OFFICER (with an honours degree in chemistry or agricultural chemistry, A.R.I.C. or an equivalent qualification, and particularly interested in analytical chemistry) IN THE CHEMISTRY DEPARTMENT, to take part in researches on the composition of milk—The Secretary, National Institute for Research in Dairying, Shinfield, Reading, quoting Ref. 61/32.

SENIOR LECTURER IN ORGANIC CHEMISTRY—The Registrar, The University, Leicester.

SENIOR LECTURER IN PHYSICS and a LECTURER IN PHYSICS—Clerk to the Governing Body, Northern Polytechnic, Holloway, London, N.7.

SENIOR LECTURER OR LECTURER (with a knowledge of West African livestock and feeding-stuffs, especially grass) IN AGRICULTURAL BIOCHEMISTRY AND NUTRITION at the University College, Ibadan, Nigeria—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1.

SENIOR TECHNICIAN, for histological research—The Secretary, The Royal Free Hospital, Gray's Inn Road, London, W.C.1.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

Materials for Gas Chromatography: Standards and Data for "Embaphase", Stationary Phases, and "Embace" Kieselguhr. Second edition. Pp. 68. (Dagenham: May and Baker, Ltd., 1961.) [250

The Industrial Diamond Information Bureau. Diamonds in Industry: an Introduction. Pp. 12. The Diamond Research Laboratory. Pp. 4. (London: The Industrial Diamond Information Bureau, 1961.) [250

Aviation Botany, Vol. 1, No. 1 (September, 1961). (An International Journal devoted to Plant Radiobiology and Closely Related Fields.) Pp. 100. Annual subscription rates: (A) For libraries, government establishments, research laboratories, etc., 140s.; 20 dollars. (B) For individuals who place their orders directly with the publisher and certify that the journal is for their personal use, 70s.; 10 dollars. (London and New York: Pergamon Press, 1961.) [250

Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences. No. 710, Vol. 244 (21 September, 1961): A Study of a Natural Population of *Phytoecia Oviacea* (Forster) (Coleoptera, Chrysomeloidea). By O. W. Richards and Nadejda Waloff. Pp. 205–257. (London: Royal Society, 1961.) 18s. [250

Falklands Islands Dependencies Survey. Scientific Reports. No. 28: The Geology of the South Shetland Islands. 1: The Petrology of King George Island. Pp. 28+3 plates. 10s. net. No. 27: The Geology of the South Shetlands Islands. 2: The Geology and Petrology of Deception Island. By D. D. Hawkes. Pp. 43+4 plates. 16s. net. (London: H.M. Stationery Office, 1961. Published for the Colonial Office.) [250

Other Countries

American Academy of Arts and Sciences, Boston. Records of the Academy (including new members elected 10 May, 1961), 1960–1961. Pp. 157. (Boston, Mass.: American Academy of Arts and Sciences, 1961.) [250

United States Department of the Interior: Geological Survey. Bulletin 1058–H: Geology of Part of the Craig C–2 Quadrangle and Adjoining Areas, Prince of Wales Island, Southeastern Alaska. By C. L. Sainsbury. Pp. v+299–362+plates 33–39. Bulletin 1087–I: Relation of Uranium Deposits to Tectonic Pattern of the Central Cordilleran Foreland. By Frank W. Osterwald and Basil G. Dean. Pp. iii+337–390+plates 28–34. Bulletin 1116–E: Index to Geophysical Abstracts 180–183, 1960. By James W. Clarke, Dorothy B. Vitaliano, Virginia S. Neuschel, and others. Pp. ii+637–695. 25 cents. Bulletin 1117–A: Geochemistry of Minor Elements on Coals of the Northern Great Plains Coal Province. By Peter Zubovic, T. Stadnichenko, and Nola B. Sheffey. Pp. iv+58+plate 1. 50 cents. (Washington, D.C.: Government Printing Office, 1961.) [250

United States Department of the Interior: Geological Survey. Water-Supply Paper 1498–D: Effect of Depth of Flow on Discharge of Bed Material. By Bruce R. Colby. Pp. iii+12. 15 cents. Water-Supply Paper 1522: Quality of Surface Waters of the United States, 1957. Parts 7 and 8: Lower Mississippi River Basin and Western Gulf of Mexico Basins. Prepared under the direction of S. K. Love. Pp. x+499. Water-Supply Paper 1539–C: Ground-Water Reconnaissance of Winnemucca Lake Valley, Pershing and Washoe Counties, Nevada. By C. P. Zones. Pp. iii+18+plate 1. 45 cents. Water-Supply Paper 1539–R: Selected Bibliography on Evaporation and Transpiration. By T. W. Robinson and A. I. Johnson. Pp. ii+25. Water-Supply Paper 1581: Ground-Water Potentialities in the Crescent Valley, Eureka and Lander Counties, Nevada. By C. P. Zones. Pp. iv+50+plate 1. Water-Supply Paper 1610–A: Waterpower Resources of the Bradley River Basin, Kenai Peninsula, Alaska. By F. A. Johnson. Pp. iv+25+plate 1. (Washington, D.C.: Government Printing Office, 1961.) [250

Canada. National Research Council, and Medical Research Council. Report on University Support, 1960–61. Pp. 123. (N.R.C. No. 6390.) (Ottawa: National Research Council, and Medical Research Council, 1961.) [250

Tokyo Astronomical Observatory. Tokyo Astronomical Bulletin, Second Series. No. 123, (January 25, 1961): A Total Eclipse of the Sun, July 20, 1963 and its Circumstances in Hokkaido, Japan. By Shinko Aoki and Yoshiko Endo. Pp. 1695–1706. No. 140, (February 20, 1961): A Method of Improvement of the PZT Star Places. By Masahisa Torao, Shigetaka Iijima, and Naoyuki Matsunami. Pp. 1759–1769. No. 142, (March 5, 1961): Polar Tube Observations during 1960. By Baosuke Sekiguchi and Jun'itsu Matsumoto. Pp. 1901–1902. No. 143, (March 20, 1961): On the Auto-Compensating Effect in Travel Time of Radio Signals. By Shigetaka Iijima. Pp. 1903–1916. No. 144, (February 5, 1961): A New Photo-electric Photometer with Electronic Refrigerator. By Masaaki Huruhata and Masatoshi Kitamura. Pp. 1917–1922. (Tokyo: Tokyo Astronomical Observatory, 1961.) [250

Occasional Papers of the California Academy of Sciences. No. 30, (August 31, 1961): The Immature Stages of *Callomyia*, with the description of a New Species of this Genus (Diptera: Platypezidæ). By Edward L. Kessel. Pp. 10+1 plate. (San Francisco: California Academy of Sciences, 1961.) [250

Annals of the New York Academy of Sciences. Vol. 94, Article 1: Plasma Proteins in Health and Disease. By George T. Dimopoullos and 40 other authors. Pp. 1–336. (New York: New York Academy of Sciences, 1961.) [250

National Academy of Sciences: National Research Council. Nuclear Science Series. NAS-NS 3038: The Radiochemistry of Tellurium. By G. W. Leddicotte. Pp. v+42. (Washington, D.C.: Office of Technical Services, Department of Commerce, 1961.) 50 cents. [250

Canada: Department of Mines and Technical Surveys. Geological Survey of Canada. Geological Maps—Preliminary Series. Map 9–1961: Kettle Rapids, Manitoba, (Sheet 54D). Map 10–1961: La Loche, Saskatchewan, (Sheet 74C). Map 16–1961: Firebag River Area, Alberta and Saskatchewan, (Sheets 74E (E½) and 74F (W½)). Geological Maps. Map 1068–A: Collins Point, West of Principal Meridian, Manitoba, (Sheet 63 N/3, West Half.) (Ottawa: Geological Survey of Canada, 1961.) [250

Administration of South-West Africa. Marine Research Laboratory. Investigational Report No. 2: The Pilchard of South-West Africa (*Sardinops ocellata*)—Age Studies and Age Composition of *Sardinops ocellata* in the Commercial Catches, 1952–1958, and a New Method for the Determination of the Age of *Sardinops ocellata*. By O. Nawratil. Pp. 43. (Windhoek: Secretary for South-West Africa, 1961.) [250

United States Department of Agriculture. Farmers' Bulletin No. 1893: Control of Grape Diseases and Insects in the Eastern United States. By J. R. McGrew, G. W. Still and Howard Baker. Pp. 1+32. (Washington, D.C.: Government Printing Office, 1961.) 20 cents. [250

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